

live attenuated bacteria of the species *Actinobacillus pleuropneumoniae*.

The present invention relates to live attenuated bacteria of the genus *Actinobacillus pleuropneumoniae*, having a mutation in a gene encoding a toxin, methods for the production of such bacteria, to vaccines comprising such bacteria, methods for the production of such vaccines, to vaccines comprising a toxin, methods for the production of such vaccines and methods for the protection of animals against infection with bacteria of the genus *Actinobacillus pleuropneumoniae*.

Bacteria belonging to the genus *Actinobacillus* all produce so-called RTX-toxins. (RTX stands for repeat in toxin).

It is the presence of these RTX-toxins that highly contributes to the pathogenic character of these bacteria.

The RTX-toxins have been extensively reviewed by Braun et al. (Critical Rev. in Microbiol. 18(2): 115-158 (1991)). RTX-toxins in Gram-negative strains have also been reviewed in Welch, R.A. (Molecular Microbiology 5/3: 521-528 (1991)) and in Welch et al. (Inf. Agents and Disease 4: 254-272 (1995)).

All known RTX-toxins display some kind of cytotoxic or cytolytic activity. The target-cell and host-specificity differ however, depending on the toxin and on differences in acylation (McWhinney et al.; J. Bact. 174: 291-297 (1992) and Hackett et al.; J. Biol. Chem. 270: 20250-20253 (1995)). As a result of the difference in target cells, the various toxins of the RTX-toxin family are known e.g. as haemolysin, cytolsin or cytotoxin.

The genus *Actinobacillus* comprises a number of different species, inter alia, *Actinobacillus pleuropneumoniae*, *A. actinomycetemcomitans*, *A. suis*, *A. rossii*, *A. equuli* and *A. lignieresii*.

Actinobacillus pleuropneumoniae produces serotype-dependent RTX-toxins that are cytotoxic/cytolytic to pig, horse, bovine and human erythrocytes, to rabbit and porcine neutrophils and to porcine alveolar macrophages. (Rosendal et al; Am. J. Vet. Res. 49: 1053-1058 (1988), Maudsley J.R. and Kadis S; Can. J. Microbiol. 32: 801-805 (1986), Frey, J and Nicolet, J; Inf. & Imm. 56:2570-2575 (1988), Bendixon et al; Inf. & Imm. 33: 673-676 (1981), Kamp, E.M. and van Leengoed, L.A.M.G.; J. Clin. Microbiol. 27: 1187-1191 (1989)).

Infections with *Actinobacillus* in pigs are the cause of severe economic losses to pig industry, due to acute mortality in young pigs and reduced weight gain in older animals.

The genetic organisation of the operons involved in the synthesis, activation and transportation of the RTX toxins in Gram-negative bacteria has been reviewed recently by Coote, J.G. (FEMS Microbiology reviews 88: 137-162 (1992)). Frey has specifically reviewed the known three RTX-toxins in *Actinobacillus pleuropneumoniae* in Bacterial Protein Toxins, Zbl Bakt. Suppl. 24, p. 322-, Freer et al. (Eds.), Gustaf Fischer, Stuttgart, Jena, New York, 1994.

In *Actinobacillus pleuropneumoniae*, this kind of RTX-operon contains four genes: the actual Toxin-gene (A), an Activator-gene (C), and two genes (B and D) encoding proteins involved in secretion of the toxin into the surrounding medium. The primary translation-product of the Toxin-gene (A) is a non-toxic protein, of which the toxic activity is activated by the Activator-gene (C) product.

Until recently, it was assumed that only three RTX-toxins, all having the above-described genetic organisation or at least having the Toxin-gene (A) and Activator-gene (C), existed in *Actinobacillus* species.

These three RTX-toxins, ApxI, Apx-II and Apx-III have respectively a pronounced haemolytic activity (ApxI), a mild haemolytic activity (Apx-II) or a macrophage-cytotoxic activity (Apx-III).

The various toxic activities are fairly randomly divided over the serotypes. There are four subgroups:

- a subgroup A, represented by serotypes 1, 5, 9 and 11, producing ApxI and Apx-II,
- a subgroup B, represented by serotypes 2, 3, 4, 6 and 8, producing Apx-II and Apx-III,
- a subgroup C, represented by serotype 10, producing ApxI only,
- a subgroup D, represented by serotype 7 and 12, producing Apx-II only,

It is known, that ApxI, -II, and -III all are essential elements in universal vaccines against *Actinobacillus pleuropneumoniae* infection: a vaccine not comprising at least ApxI, -II, and -III will not provide protection against all *Actinobacillus pleuropneumoniae* serotypes. Also, a vaccine not comprising at least the Apx-toxins of one specific serotype will not even induce protection against that single serotype.

Subunit vaccines based on in vitro synthesised RTX-toxins from *A. pleuropneumoniae* that lost their toxicity have been described earlier, e.g. in European Patent EP No. 0.354.628, in which subunit vaccines based upon a haemolysin and a cytotoxin of *A. pleuropneumoniae* are disclosed, and in European Patent EP No 0.453.024, in which *A. pleuropneumoniae* subunit vaccines based upon haemolysins, cytotoxins and outer membrane proteins are disclosed.

There are however four important disadvantages to subunit vaccines in general:

- high amounts of antigenic material are needed in order to adequately trigger the immune system.
- usually, only B-cell immunity is triggered.
- several protective antigens are only triggered *in vivo*, and therefore can not be present in subunit vaccines.
- a live pathogenic bacterium has many important immunogenic molecules, such as Outer Membrane Proteins and capsular polysaccharides, all potentially important for protection and thus to be included in an efficient subunit vaccine.

Next to the obvious problems mentioned under points one and two, especially the fourth point makes it difficult to make an efficient subunit vaccine.

This is e.g. illustrated by the *A. pleuropneumoniae* subunit vaccine disclosed in European Patent EP No 0.453.024 mentioned above, in which four different subunits (three RTX-toxins and an outer membrane protein) are combined in one vaccine.

It is clear, that in order to overcome the disadvantages of subunit vaccines against *Pasteurellaceae*-infection, a live attenuated vaccine would be highly desirable.

A live attenuated vaccine has the following advantages:

- it can be administered in low doses (it is self-replicating)
- it closely mimics the natural/wild-type infection
- it provides all the possible immunologically important antigens at the same time.

Nevertheless, in spite of the clear advantages, no live vaccines based on *Actinobacillus pleuropneumoniae* were commercially available prior to the present invention.

The reason for this lies in the following paradox: as mentioned before, ApxI, -II, and -III all are essential elements of universal vaccines against *Actinobacillus pleuropneumoniae* infection. Live vaccines therefore have to produce these three RTX-toxins. These three

RTX-toxins are however strong virulence factors in all *Actinobacillus* species (see e.g. Coote, J.G.; FEMS Microbiology reviews 88: 137-162 (1992), Tascon et al.; Mol. Microbiol. 14: 207-216 (1994)), Jansen et al.; Inf. & Imm. 63: 27-37 (1995)).

Deletion of the RTX-toxins in order to attenuate the virulence of live App strains is technically feasible, but this does not provide a solution for the dilemma: such RTX-negative strains would be useless as live attenuated vaccine strains since they do no longer induce immunity in the host against the haemolytic/cytotoxic activity of *Actinobacillus pleuropneumoniae* field strains.

Virulence factors that, although important in the induction of immunity, do play a less important role in building up immunity than ApxI, -II and -III, and thus can in principle be deleted are however currently not known.

It would thus be highly desirable to have a site on the genome of App that attributes to virulence and therefore leads to an attenuated App strain when modified, whereas at the same time it is, although useful in triggering immunity, dispensable from a vaccine point of view. No such sites are however currently known. Moreover, it would be highly desirable if such a site would be universally present in all App strains, instead of being restricted to certain serotypes. Such a site would then allow all different serotypes to be attenuated by deletion of that same site.

It is one of the objectives of the present invention to provide such an attenuation site, universally present in all *Actinobacillus pleuropneumoniae* strains regardless their serotype.

Recently, a new gene was found in a serotype 1 strain of *Actinobacillus pleuropneumoniae* (Thesis T.J. Anderson Nov. 1995).

Although this gene does not resemble the known *Actinobacillus* ApxI, -II and -III genes, it bears resemblance to RTX-toxin genes known from bacteria belonging to *Neisseria meningitidis*, for which reason it was named RTX-gene *apxIV*. The gene however differs in almost all aspects from the three known RTX-toxin genes *apxI*, -II and -III present in the various species of the *Actinobacillus* family as described above. First of all, the genomic organisation is completely different. Secondly, there is no activator-mechanism as is found for the known Apx-toxins. In the third place, no specific *in vivo* haemolytic or cytotoxic activity could at that time be attributed to the gene, or it's possible gene product.

It was now surprisingly found that this gene, fully in contrast with the three known RTX-genes, is present in all bacteria of the species *Actinobacillus pleuropneumoniae*, regardless their serotype. This was determined by hybridisation of a probe comprising *apxIV* coding sequences with restriction fragments of the DNA from *Actinobacillus pleuropneumoniae* of all serotypes as described in Example 6 and 7.

Unexpectedly it was found now, that *apxIV* deletion mutants are viable, but they behave less virulent compared to their *apxIV*-possessing parent strains.

Therefore, it was determined that the gene product, the ApxIV toxin is a virulence factor in all *Actinobacillus pleuropneumoniae* strains. This is an unexpected conclusion, since up until now, no effects at all, let alone effects possibly influencing virulence had been attributed to the gene product *in vivo*. In fact, up until now there was not even proof that the gene was expressed in *Actinobacillus pleuropneumoniae in vivo* or *in vitro* anyway.

It therefore is one of the merits of the invention that it was found that:

- the *apxIV* gene is present in all *A. pleuropneumoniae* strains regardless the serotype,
- the *apxIV* gene product is a virulence factor in all *A. pleuropneumoniae* serotypes,
- *A. pleuropneumoniae* strains with a deletion in the *apxIV* gene are still viable but have a decreased virulence without significantly impairing the immunogenic properties of the strains,

Therefore, the invention provides for the first time live attenuated bacteria of the species *Actinobacillus pleuropneumoniae*, that do not produce a functional ApxIV toxin.

A functional ApxIV toxin is considered to be a protein that has all the characteristics of the ApxIV toxin as expressed in a wild-type bacterium, and is expressed at the wild-type level. Therefore, a non-functional ApxIV toxin is considered to be a toxin that lacks some or all of the characteristics of the ApxIV toxin as expressed in a wild-type bacterium, and/or is expressed at a level, insufficient to obtain wild-type effects of ApxIV toxin.

The inability to produce the ApxIV toxin can e.g. be due to modifications in the coding sequence encoding the ApxIV toxin. It may also be e.g. the result of modifications in regions known to be involved in transcription of the *apxIV* gene, such as the promoter region, or of modifications in regions involved in translation, such as the ribosome binding site.

The overall structure of the *apxIV* gene is given in figure 1.

In this figure, the direct repeat regions, characteristic for ApxIV toxin are indicated by dashed boxes, whereas the also ApxIV-specific glycine-rich nonapeptide regions are indicated by black arrows. The repeats are found at the C-terminal part of ApxIV. These characteristic features are present in all *Actinobacillus pleuropneumoniae* serotypes. The nucleic acid sequence and amino acid sequence of two serotypes are represented in SEQ. ID. No. 1-4. SEQ. ID. NO. 1 shows the nucleic acid sequence of the *apxIV* gene of App serotype 1, and SEQ. ID. NO. 2 shows the matching amino acid sequence of the serotype 1 ApxIV toxin. SEQ. ID. NO. 3 shows the nucleotide sequence of the *apxIV* gene of App serotype 3, whereas SEQ. ID. NO. 4 shows the matching amino acid sequence of the serotype 3 ApxIV toxin. Figure 2 shows the strikingly high level of conservation at amino acid level, especially in the N-terminal 650 amino acids, between the Apx-toxins of the various *Actinobacillus pleuropneumoniae* serotypes. This is also a remarkable characteristic of the *apxIV* genes. It is clear from figure 1, that a variation in the number of repeats at the C-terminal part of the toxin may occur, depending on the serotype. This variation accounts for the difference in size of the genes and encoded toxins obtained from the various serotypes.

There may be some variation in nucleic acid sequence even between *apxIV* genes isolated from different isolates of *Actinobacillus pleuropneumoniae*, belonging to the same serotype. This is due to natural variation well known in the art to exist in all organisms. It is possible that some amino acids in the ApxIV toxin encoded by the *apxIV* gene are replaced by others in the ApxIV toxin of another serotype, while the polypeptide is not altered in its function. For instance, a polypeptide containing Asp at a certain site, and its variant containing Asn at the comparable site still have the same properties. This process in which an amino acid is replaced by an functionally analogous amino-acid is called functional displacement. In this case the variant proteins are called functional variants.

Another cause of variation is the phenomenon of degeneracy of the genetic code. Shortly, it means, that e.g. the amino acid glutamic acid is coded for by both GAT and GAA. This phenomenon holds for all amino acids, except Met and Trp. Thus, it is obvious, that e.g. the ApxIV toxin of serotype 1, as given in the present invention can not only be coded for by the nucleotide sequence given in SEQ ID NO: 1 but also by a very large variety of other sequences, still all giving the same or functionally the same polypeptides.

Therefore, a variant *apxIV* sequence encoding a polypeptide that is functionally comparable to the *Ap_{xIV}* toxin falls within the scope of the present invention.

Live attenuated bacteria according to the invention can be obtained in several ways. One possible way of obtaining such bacteria is by means of classical methods such as the treatment of wild-type *Actinobacillus pleuropneumoniae* bacteria with mutagenic agents such as base analogues, treatment with ultraviolet light or temperature treatment. Strains that do not produce a functional *Ap_{xIV}* toxin do not or to a lesser extent induce anti-*Ap_{xIV}* toxin antibodies, and therefore can easily be selected in animal tests. The necessary antiserum can be obtained as described below in Example 3. Another possibility is to deliberately introduce, using recombinant DNA-technology, a well-defined mutation in the gene encoding the *Ap_{xIV}* toxin. Such a mutation may be an insertion, a deletion, a replacement of one nucleotide by another one or a combination thereof, with the only proviso that the mutated gene no longer encodes a functional *Ap_{xIV}* toxin. It can easily be seen, that especially mutations introducing a stop-codon in the open reading frame, or mutations causing a frame-shift in the open reading frame are very suitable to obtain a strain which no longer encodes a functional *Ap_{xIV}* toxin. Such mutations can e.g. be made by means of in vitro site directed mutagenesis using the Transformer® kit sold by Clontech. Many other standard recombinant DNA techniques such as digestion of the gene with a restriction enzyme, followed by endonuclease treatment and religation, are equally applicable.

Therefore, in a preferred form, this embodiment of the invention relates to live attenuated bacteria in which the gene encoding the *Ap_{xIV}* toxin comprises a mutation.

Well-defined mutations involving the deletion of fragments of the *apxIV* gene or even the whole gene, or the insertion of heterologous DNA-fragments, when compared to classically induced mutations, have the advantage that they will not revert to the wild-type situation.

Thus, in a more preferred form, this embodiment of the invention refers to live attenuated bacteria in which the gene encoding the *Ap_{xIV}* toxin comprises an insertion and/or a deletion.

Given the large amount of vaccines given nowadays to pigs, it is clear that combined administration of several vaccines would be desirable, if only for reasons of decreased vaccination costs. It is therefore very attractive to use live attenuated vaccine strains as a recombinant carrier for heterologous genes, encoding antigens selected from other pathogenic micro-organisms or viruses. Administration of such a recombinant carrier has the advantage that after administration of such a carrier, immunity is induced against two or more diseases at the same time. The live attenuated bacteria according to the present invention provide a very suitable carrier for heterologous genes, since the gene encoding the ApxIV toxin can be used as an insertion site for such heterologous genes. The use of the *apxIV* gene as an insertion site has the advantage that at the same time the *apxIV* gene is inactivated, and the newly introduced heterologous gene can be expressed in accordance with the homologous *Actinobacillus pleuropneumoniae* genes. The construction of such recombinant carriers can be done routinely, using standard molecular biology techniques such as homologous recombination. Therefore, in an even more preferred embodiment, the present invention relates to live attenuated bacteria of the species *Actinobacillus pleuropneumoniae* that do not produce a functional ApxIV toxin, and in which there is a heterologous gene inserted in the *apxIV* gene. Such a heterologous gene can, as mentioned above, e.g. be a gene encoding an antigen selected from other pathogenic micro-organisms or viruses. Another possibility is to insert a gene encoding a protein involved in triggering the immune system, such as an interleukine or an interferone.

In a still even more preferred form of the invention, the heterologous gene encodes one or more antigens selected from the group consisting of Porcine Reproductive Respiratory Syndrome (PRRS) virus, Pseudorabies virus, Porcine Influenza virus, Porcine Parvovirus, Transmissible Gastroenteritis virus, rotavirus, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis* and *Streptococcus suis*.

There is however a serious pitfall in expression of heterologous genes in recombinant carriers: it is known that several proteins are toxic if they are expressed in heterologous bacteria. Therefore, genes encoding such proteins can never be introduced in heterologous carriers, since successful recombinants will eventually die as a result of the expression a certain amount of the heterologous gene. The P2-protein of *Haemophilus influenzae*, to

name just one example, can simply not be expressed in *E. coli*. (Munson et al., Infect. & Immun. 57: 88-94 (1989)). It is one of the objectives of the present invention to offer a recombinant live carrier that does not have this drawback. It was unexpectedly found, that although the *apxIV* gene is efficiently expressed *in vivo* (see Example 5), it is not expressed *in vitro* (see Example 4). This was concluded from the failure to show the presence of ApxIV toxin in *in vitro* grown *A. pleuropneumoniae* cultures. This means that ApxIV expression is switched on or off, depending on the environment in which *A. pleuropneumoniae* is grown. This feature offers an unexpected advantage over known live recombinant carriers: if the expression of the heterologous gene is brought under the control of the *apxIV* promoter, the live attenuated *P. pleuropneumoniae* carrier according to the invention can be grown *in vitro* to high densities, regardless the inserted heterologous gene, since the foreign gene will not be expressed under these conditions. After administering a number of bacteria to the host, the expression of the heterologous gene will start and at some time during replication or after the death of the bacterium it will become available to the immune system of the host. The heterologous gene to be expressed can be functionally linked to the *apxIV* promoter by e.g. replacing the coding sequence of the *apxIV* gene by the coding region of the heterologous gene. It is not necessary to replace the whole *apxIV* gene: it suffices to replace the ATG-codon of ApxIV by the coding region of the heterologous gene including its stop-codon. It is also possible to express a heterologous gene under the influence of the *apxIV* promoter by making a fusion construct. This can be made by inserting the heterologous gene in frame with the *apxIV* reading frame downstream of the *apxIV* ATG codon.

The wording "functionally linked to the *apxIV* promoter" means that transcription of the heterologous gene starts at the *apxIV* promoter.

It goes without saying that each location of the inserted heterologous gene in which it is functionally linked to the *apxIV* promoter falls within the scope of the invention.

Therefore, the most preferred form of this embodiment relates to live attenuated bacteria according to the present invention, carrying a heterologous gene that is functionally linked to the promoter region of the *apxIV* gene.

The surprising finding that the native *apxIV* promoter is a switchable promoter that is switched off *in vitro* and switched on *in vivo* makes this promoter a very versatile expres-

sion tool both in its natural host and as a heterologous promotor in other bacteria. When used as a heterologous promotor in other bacteria, the DNA comprising the promotor can be isolated from its host and transferred to a bacterium other than *Actinobacillus pleuropneumoniae*. Another option that has now become feasible, is the cloning of several copies of the *apxIV* promotor each controlling the expression of another gene. This can be done in the host bacterium *Actinobacillus pleuropneumoniae*, but this principle of multiple copies is equally applicable to other bacteria. As mentioned above, the promotor can be used for the selective *in vivo* expression of one or more heterologous genes encoding antigens selected from other pathogenic micro-organisms or viruses. The promotor can also be used for the expression of a heterologous DNA sequence encoding a cytokine such as an interleukin, Tumor Necrosis Factor or an interferon. Several cytokines, e.g. interferons are known to play an important role as immune modulators. Thus it may be advantageous to express such genetic information under the control of the *apxIV* promotor. Therefore, another embodiment of the invention relates to a nucleotide sequence harbouring the promotor controlling the expression of the *apxIV* gene.

The switchable promotor that in the native situation controls the expression of the *apxIV* gene, was now found to be located in the DNA fragment between position 451 and 1132 of SEQ ID NO: 5. It is clear, that those parts of this DNA fragment that are not essential promotor elements need not necessarily be present in the fragment. Thus, shorter fragments of this DNA fragment in which the promotor activity is retained, are equally suitable for the expression of heterologous genes. Therefore, a more preferred form of this embodiment relates to a nucleotide sequence comprising the DNA fragment from position 451 to 1132 of SEQ ID NO: 5 or a subfragment thereof still having promotor activity.

Bacterial promotors all share two consensus regions, the so-called -10 and the -35 region. Although the flanking sequence of these consensus regions may to a certain extent influence the efficiency of the promotor, it can be advantageous to use only that part of the promotor region that comprises the DNA fragment between -35 and the ATG codon. This DNA fragment is located between position 617 and position 641 of SEQ ID NO: 5. Therefore, in a more preferred form of this embodiment the invention relates to a nucleotide sequence comprising the DNA fragment from position 617 to 641 of SEQ ID NO: 5.

The present invention also relates to *ApxIV* toxin as a subunit vaccine component.

Subunit vaccines will most probably comprise the three known Apx-toxins. This was mentioned above. Since it was unexpectedly found, that the ApxIV toxin is however present in all *A. pleuropneumoniae* serotypes as mentioned above, it is a desirable additional component of subunit vaccines: neutralising antibodies raised against the ApxIV toxin provide protection against the ApxIV toxin produced by each and every *Actinobacillus pleuropneumoniae* strain, regardless the serotype. Therefore, another embodiment of the invention relates to subunit vaccines for the protection of animals against infection with a bacterium of the species *Actinobacillus pleuropneumoniae*, that comprise purified ApxIV toxin. The ApxIV toxin can be administered alone, or in combination with any or all of the toxins ApxI, -II and -III mentioned above and/or e.g. in combination with Outer Membrane Proteins (OMPs) of *Actinobacillus pleuropneumoniae*. Such vaccines can easily be prepared by admixing ApxIV toxin in an amount sufficient to induce an immune response, and a pharmaceutically acceptable carrier. Production of the ApxIV toxin is possible by introducing the *apxIV* gene in a suitable expression vector, expression of the gene and isolation of the toxin. Many versatile expression systems are known in the art, such as bacterial expression systems, baculovirus expression systems and mammalian cell expression systems. In Example 3 it is described how to obtain the ApxIV toxin by expression of the gene in *E. coli*.

Still another embodiment of the invention relates to live attenuated vaccines comprising live attenuated bacteria as described above for the protection of animals against infection with a bacterium of the species *Actinobacillus pleuropneumoniae*. Such vaccines can be obtained by admixing live attenuated bacteria with a pharmaceutically acceptable carrier. These vaccines comprise at least an immunogenically effective amount of the live attenuated producing bacterium according to the invention. Immunogenically effective means that the amount of live attenuated bacterium administered at the moment of vaccination is sufficient to induce in the host an effective immune response to virulent forms of the RTX-toxin producing bacterium. The useful dosage to be administered will vary depending on the age, weight and animal vaccinated, the mode of administration and the type of pathogen against which vaccination is sought. The vaccine may comprise any dose of bacteria, sufficient to evoke an immune response. Doses ranging between 10^3 and 10^{10} bacteria are e.g. very suitable doses.

The pharmaceutically acceptable carrier may be as simple as water, but it may e.g. also comprise culture fluid in which the bacteria were cultured. Another suitable carrier is e.g. a solution of physiological salt concentration. Other examples of pharmaceutically acceptable carriers or diluents useful in the present invention include stabilisers such as SPGA, carbohydrates (e.g. sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein, protein containing agents such as bovine serum or skimmed milk and buffers (e.g. phosphate buffer).

Optionally, one or more compounds having adjuvant activity may be added to the vaccine. Adjuvantia are non-specific stimulators of the immune system. They enhance the immune response of the host to the invading pathogen. Examples of adjuvantia known in the art are Freund's Complete and Incomplete adjuvants, vitamin E, non-ionic block polymers, muramyl dipeptides, ISCOMs (immune stimulating complexes, cf. for instance European Patent EP-109942), Saponins, mineral oil, vegetable oil, and Carbopol (a homopolymer). Adjuvantia, specially suitable for mucosal application are e.g. the E. coli heat-labile toxin (LT) or Cholera toxin (CT).

Other suitable adjuvants are for example aluminium hydroxide, phosphate or oxide, oil-emulsions (e.g. of Bayol F (R) or Marcol 52 (R), saponins or vitamin-E solubilisate.

Therefore, in a preferred form, the vaccines according to the present invention comprise an adjuvant.

For administration to animals, the vaccine according to the presentation can be given inter alia intranasally, intradermally, subcutaneously, by aerosol or intramuscularly.

There are several ways to store both subunits and live organisms. Storage in a refrigerator is e.g. a well-known method. Also often used is storage at -70°C in a buffer containing glycerol. Bacteria can also be kept in liquid nitrogen. Freeze-drying is another way of conservation. Freeze-dried bacteria can be stored and kept viable for many years. Storage temperatures for freeze-dried bacteria may well be above zero degrees, without being detrimental to the viability. Freeze-drying is equally applicable for subunits.

Freeze-drying can be done according to all well-known standard freeze-drying procedures. Optional beneficial additives, such as e.g. skimmed milk, trehalose, gelatin or bovine serum albumin can be added in the freeze-drying process.

Therefore, in a more preferred embodiment, the vaccine according to the present invention is in a freeze-dried form.

In an even more preferred form of this embodiment, the vaccine additionally comprises one or more antigens selected from other pathogenic micro-organisms or viruses.

Such a vaccine can be obtained by adding one or more antigens selected from other pathogenic bacteria or viruses to the live attenuated bacterium according to the invention and a pharmaceutically acceptable carrier as described above.

Of course, it is possible to add not only one or more antigens, but also one or more of the whole pathogens as such, in an inactivated or live form.

It can alternatively be obtained by cloning the genetic information encoding one or more antigens selected from other pathogenic micro-organisms or viruses into the live attenuated bacterium, using known recombinant DNA technology as described above.

Such vaccines are of course less stressing for the animal to be vaccinated than separate vaccinations with each of the pathogens, both from a medical and a physical point of view.

In a still even more preferred form, these antigens are selected from the group consisting of Porcine Reproductive Respiratory Syndrome (PRRS) virus, Pseudo-rabies virus, Porcine Influenza virus, Porcine Parvovirus, Transmissible Gastroenteritis virus, rotavirus, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis* and *Streptococcus suis*.

The invention also relates to methods for the preparation of a live attenuated bacterium of the species *Actinobacillus pleuropneumoniae* that is not capable of producing a functional ApxIV toxin. These method comprise the introduction of a mutation in the gene encoding the apxIV protein. Both classical mutation techniques, using mutagenic agents, and recombinant DNA techniques well-known in the art for insertion, replacement or deletion of genetic information from the apxIV gene are applicable.

In a preferred form, the above mentioned methods are used for the introduction of a deletion.

Methods for the preparation of a live attenuated vaccine according to the invention, that comprise admixing bacteria according to the invention with a pharmaceutically acceptable carrier are also part of the invention.

Also falling within the scope of the invention are methods for the preparation of a subunit vaccine. Such methods comprise the mixing of purified ApxIV toxin with a pharmaceutically acceptable carrier.

Another generally acknowledged problem in the field of vaccination with live vaccines is the following: the presence of antibodies against a certain pathogen in the serum of a host animal indicates that the host has been infected with the pathogen, either in a virulent or attenuated form. It is however impossible to discriminate between field-infected animals and animals vaccinated with a live vaccine strain. The live attenuated *Actinobacillus pleuropneumoniae* according to the present invention offers a solution to this problem as follows:

As described in Example 3, the *apxIV* gene of *Actinobacillus pleuropneumoniae* serotype 1 has been isolated and expressed in a heterologous host cell. This expression product was subjected to PAGE-gel electrophoresis and then used for Western-blotting. The blots were incubated with convalescent serum obtained from a deliberately *Actinobacillus pleuropneumoniae*-infected pigs, and sera from field-strains. It was found, that the *apxIV* gene is expressed *in vivo* in all *Actinobacillus pleuropneumoniae* field strains tested. This implicates, that pigs infected with *Actinobacillus pleuropneumoniae* will always have antibodies against the strain with which they were infected, regardless the serotype of the infectious strain.

The live attenuated bacteria according to the present invention can, due to the deletion of the *apxIV* gene, no longer make ApxIV toxin. Therefore animals vaccinated with a live attenuated *Actinobacillus pleuropneumoniae* strain according to the invention will not have antibodies against ApxIV toxin in their serum.

In a comparative test, e.g. an ELISA test, such sera will therefore react with all immunogenic *Actinobacillus pleuropneumoniae*-proteins such as e.g. ApxI, II and/or III, but not

with ApxIV. Sera from pigs infected with an *Actinobacillus pleuropneumoniae* field strain however will react with all immunogenic *Actinobacillus pleuropneumoniae*-proteins, including ApxIV. Therefore, the live attenuated *Actinobacillus pleuropneumoniae* according to the present invention turns out to be a very suitable marker vaccine, i.e. a vaccine strain that can be discriminated from a field strain.

A diagnostic test for the discrimination between vaccine strains and field strains can be a simple ELISA-test in which purified ApxIV toxin is coated to the wall of the wells of an ELISA-plate. Incubation with serum from pigs to be tested, followed by e.g. incubation with a labelled anti-pig antibody can then reveal the presence or absence of antibodies against ApxIV toxin.

Another example of a diagnostic test system is e.g. the incubation of a Western blot comprising purified ApxIV toxin with serum of pigs to be tested, followed by detection of specific anti-ApxIV antibodies.

Therefore, diagnostic test for the discrimination between sera from pigs infected with *Actinobacillus pleuropneumoniae* field strains and from pigs vaccinated with a vaccine comprising live attenuated vaccine *Actinobacillus pleuropneumoniae* strains according to the invention, that comprise purified ApxIV toxin, also fall within the scope of the invention.

Still another problem seen in pig health care is the following: It is difficult to determine in a both quick and unambiguous manner if a pig is infected with *Actinobacillus pleuropneumoniae* or *A. suis*, or possibly a combination of both. Diagnostic tests for the specific detection of *A. suis* are currently not available. This is mainly due to the fact that *A. pleuropneumoniae* and *A. suis* share so many antigens. As an example may serve, that two highly antigenic Apx-toxins; ApxI and ApxII have highly conserved homologues in e.g. *A. suis* (Van Ostaayen et al., submitted for publication).

The known RTX-genes, encoding the ApxI, -II and -III toxins or homologues are found in practically all members of the genus *Actinobacillus*, such as *A. pleuropneumoniae*, *A. suis*, *A. rossii* and *A. equuli*. Thus, it was initially assumed by the inventors, that the new RTX-toxin ApxIV would also be common to all members of the genus *Actinobacillus*.

It was however found after testing a the swine-pathogenic *Actinobacillus*, again surprisingly in contrast with the known three RTX-genes, that this novel RTX-gene *apxIV* is only present in the swine-pathogen *Actinobacillus pleuropneumoniae*. It is absent in all other

common swine pathogenic *Actinobacillus* species, and therefore it is also absent in *Actinobacillus suis*. See Example 6 and 7.

Therefore, it was surprisingly noticed that the presence of antibodies against ApxIV in the serum of a pig is a quick and unambiguous proof that the pig has been infected with *A. pleuropneumoniae*, and not with *A. suis* or any other swine-pathogen *Actinobacillus* species.

Thus the present invention also provides a diagnostic test based on the presence or absence of antibodies against ApxIV, and therefore a discriminating test for specifically distinguishing an infection with *A. pleuropneumoniae* from an infection with *A. suis*

Such a test can e.g. be an ELISA test that comprises in separate wells the ApxI and -II toxins, present in both *A. pleuropneumoniae* and *A. suis* and the purified ApxIV toxin. Serum from *A. suis*-infected animals will react only with the wells comprising the ApxI and -II whereas *A. pleuropneumoniae*-infected animals will also react with the well comprising the purified ApxIV toxin.

Example 1:

Cloning and analysis of the *apxIV* gene of *A. pleuropneumoniae* serotype 1.

Standard molecular biological procedures (plasmid DNA isolation, restriction digestion, agarose gel electrophoresis, Southern blotting, ligation, transformation, electroporation) were, unless stated otherwise, essentially performed as described in Sambrook et al. (Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, N.Y., 1989) or Ausubel et al., (Current Protocols in Molecular Biology. John Wiley & Sons, N.Y., 1987). PCR was performed essentially as described in Innis et al., (PCR protocols, A guide to Methods and Applications, Academic Press Inc., San Diego, 1990). Chromosomal DNA isolation was performed according to Pitcher et al., (Lett. Appl. Microbiol., 8;151-156, 1989). The origin of all *A. pleuropneumoniae* reference strains (serotype 1: strain 4074; serotype 2: strain S1536; serotype 3: S1421; serotype 4 M62; serotype 5a: K17; serotype 5b: L20; serotype 6: femø; serotype 7: WF83; serotype 8: 405; serotype 9: CVI13261; serotype 10: 13039; serotype 11: 56153 and serotype 12: 8329) is described by Frey and Nicolet, (J. Clin. Microbiol., 28;232-236, 1990). *A. pleuropneumoniae* serotype 3 strain HV114 is a field isolate (i.e. one of the serotype 3 strains tested in Beck et al., J. Clin. Microbiol., 32;2749-2754, 1994). Other *Actinobacillus* strains used; *A. rossii*: ATCC 27072; *A. equuli*: ATCC

19392; *A. suis*: ATCC 15558. *Pasteurella haemolytica* type 1 strain ATCC 14003 was used.

E. coli host strains used: XL1-blue (Stratagene, La Yolla, Ca; genotype: *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lac^fZDM15 Tn10 (Tet^r)*]) and HMS174(DE3) (AMS Biotechnology Ltd, Switzerland; genotype: F' *recA (r_{K12}- m_{K12}+) rif* IDE3).

On the basis of the preliminary sequence data obtained from the thesis of T.J. Anderson (University of Guelph, 1995), two primers, designated APXIVA-1L (5'-TGGCACTGACGGTGATGA-3') and APXIVA-1R (5'-GGCCATCGACTCA⁹ACCAT-3'), were synthesised. These primers were used in a PCR amplification, with chromosomal DNA from *A. pleuropneumoniae* serotype 3 strain HV114 and serotype 1 reference strain 4074 as a template. With both strains a fragment of 442 bp was amplified. The fragment derived from the serotype 3 chromosomal DNA was labelled with Digoxigenin-11-dUTP (Boehringer Mannheim) according to the protocol of the manufacturer (this fragment was designated probe APXIVA, see fig. 4). The labelled probe was subsequently used to hybridize a Southern blot containing *Cla*I digested chromosomal DNA from strain 4074. The probe hybridised with a fragment of approximately 8.0 kb. The *apx/V* gene from serotype 1 strain 4074 was isolated by ligating *Cla*I digested chromosomal DNA into *Cla*I digested pBluescript II SK⁻ (Stratagene USA). *E. coli* strain XL1-blue was transformed with the ligated DNA and transformants were selected on an LB plate with 100 mg/ml of ampicillin. Clones harbouring the *apx/V* were selected by colony hybridisation of a nitrocellulose replica of the plate with the APXIVA probe. Thus, a plasmid designated pROK7 was isolated and shown to harbour a *Cla*I insert of approximately 8 kb. The first 6736 bp of the *Cla*I insert were sequenced (SEQID 1) and an open reading frame of 4971 nucleotides was identified encoding a protein of 1657 amino acid residues (SEQID 2) with a predicted size of approximately 186 kD. The gene was designated *apx/V_var1* (see fig. 3).

Example 2:

Cloning and analysis of the *apx/V* gene of *A. pleuropneumoniae* serotype 3.

The labelled probe APXIVA (mentioned in example 1) was used to hybridize a Southern blot containing *Cla*I digested chromosomal DNA from strain HV114. The probe hybridised with a fragment of approximately 7.0 kb. The isolated chromosomal DNA from HV114 was

digested with *Cla*I, and ligated with *Cla*I digested pBluescript II SK⁺ (Stratagene USA). *E. coli* strain XL1-blue was transformed with the ligated DNA and transformants were selected on an LB plate with 100 mg/ml of ampicillin. Clones harbouring the *apxIV* were selected by colony hybridisation of a nitrocellulose replica of the plate with the APXIVA probe. Thus, a plasmid designated pROK5 was isolated and shown to harbour a *Cla*I insert of approximately 7 kb. The insert was analysed by sequence analysis (SEQID 3). An open reading frame of 4146 bp was identified encoding a protein of 1382 amino acid residues (SEQID 4), with a predicted size of approximately 154 kD. The gene was designated *apxIV_var3* (see fig. 3).

Example 3:

EXPRESSION OF *Ap*xIV *var3*-polyhistidine fusion proteins in *E. coli*

From plasmid pROK5, a deletion clone was made which contains the 3' end of the *apxIV* gene, starting at the *Bam*HI site (nucleotide No. 2747 in SEQ ID No: 3) up to the *Cla*I site at the end of the insert downstream of the *apxIV* gene. This plasmid was designated pROK1. Using oligonucleotides APXIVAHIS1-L (5'-AGCCATATGGGCGATTAAATTTCAG-3') and APXIVAHIS1-R (5'-TATGGATCCTCCGTGCTTCTGAGC-3') and DNA from plasmid pROK1 as a template, a DNA fragment of 2.1 kb was amplified (see fig. 4) containing the region from bp 3520 to 5643 in *apxIV_var3* (SEQID 3) flanked with *Nde*I and *Bam*HI restriction sites at the 5' and 3' end respectively. After cloning of the *Nde*I/*Bam*HI digested PCR fragment in expression vector pETHIS-1, digested with the same enzymes, a plasmid designated pJFFapxIV6/10his-1 was obtained. Plasmid pETHIS-1 is a derivative of pET14b (Novagen Inc., Madison, Wi.) where the multiple cloning site has been extended and a region encoding a histidine decamer has been inserted. Consequently, The pJFFapxIV6/10his-1 plasmid contains a translational fusion encoding a histidine hexamer, followed by amino acid residues 653 up to 1360 from SEQID 4, followed by a histidine decamer, under the control of a T7 promoter. The plasmid was transferred to *E. coli* strain HMS174(DE3) with pLysS, which contains an IPTG inducible T7 RNA polymerase gene as well as the T7 lysozyme gene for increased stability. The strain was grown in LB medium containing 25 mg/ml of chloramphenicol and 100 mg/ml of ampicillin, up to an OD₆₅₀ of 0.5, and induced with isopropyl-b-D-thiogalactopyranoside at a concentration of 10 mM. After the addition of IPTG,

the cells were incubated at 37°C for 2.5 hours, the cells were harvested by centrifugation, and fusion protein with the expected size of 80 kD was isolated in the form of inclusion bodies. The inclusion bodies were solubilized in a solution of 6M guanidine hydrochloride, 300 mM NaCl and 50 mM NaH₂PO₄ at pH 8.0 and the 80 kD fusion protein was further purified by Immobilised Metal Affinity Chromatography (IMAC) (Schmitt et al., Molecular Biology Reports 18;223-230, 1993) using Ni²⁺ chelated columns (Qiagen AG, Basel). Pure protein was eluted from the column at pH 5.0. Pooled fractions were dialysed against a solution of 300 mM NaCl and 50 mM NaH₂PO₄ at pH 8.0. A rabbit was immunised with 500 mg of the polyhistidine fusion product, mixed with 1 volume of Complete Freund's Adjuvant (Difco Labs, Detroit, MI). A booster dose of the same amount, mixed with incomplete Freund's Adjuvant was given 3 weeks later. Four weeks after the booster, the rabbit was bled and a hyperimmune serum comprising anti-ApxIV toxin antibodies, designated serum 522-409, was obtained.

Example 4:

Expression of apxIV genes in *in vitro* grown *A. pleuropneumoniae*

The *A. pleuropneumoniae* reference strain from serotype 1 was grown in Columbia broth supplemented with 10 mg/ml of b-NAD and harvested as described (Beck et al., J. Clin. Microbiol., 32;2749-2754, 1994). Adjacent to lanes comprising ApxIA, ApxIIA and ApxIVA-polyhistidine fusion proteins the concentrated culture supernatant was separated by polyacrylamide gel electrophoresis (Laemmli, Nature 227:680-685, 1970) and subjected to a Western blotting procedure (Towbin et al., Proc. Natl. Acad. Sci. USA 76:4350-4354, 1979). The Western blot was reacted with anti-ApxIA- and anti-ApxIIA monoclonal antibodies as described by Beck et al., (J. Clin. Microbiol., 32;2749-2754, 1994), and with anti-ApxIV serum 522-409 (see example 3). The isolated RTX toxin fraction of serotype 1 clearly contains ApxIA and ApxIIA. The presence of ApxIVA could not be demonstrated (see fig. 5).

Example 5:

Expression of apxIV genes in *A. pleuropneumoniae* *in vivo* during infection

A polyacrylamide gel containing the 80 kD polyhistidine-ApxIV_var3 fusion protein (see example 3) was transferred to a nitrocellulose membrane. The membrane was divided into strips which were reacted with (100-fold dilutions of) convalescent field sera against sero-

type 1 or sera from a pig, experimentally infected with the serotype 1 reference strain (Frey and Nicolet, Vet. Microbiol., 28;61-73, 1991). The reaction was visualised using alkaline phosphatase-labelled conjugate against rabbit IgG (Kirkegaard Perry Inc., Gaithersburg, Md.) and NBT (4-Nitrobluetetrazolium chloride) and BCIP (5-Bromo-4-chloro-3-indolyl-phosphate) colour development (see fig. 6). The serotype 1 field sera, as well as serum from the experimentally infected pig react with the 80 kD polyhistidine-ApxIV_var3 protein. This indicates that the ApxIV protein actually is expressed, is antigenic and induces anti-ApxIV toxin antibodies during *A. pleuropneumoniae* infection in pigs.

Example 6:

Presence of *apxIV* genes in all *A. pleuropneumoniae* serotypes and the absence thereof in non-*pleuropneumoniae* *Actinobacillus*-strains using Southern blotting

To investigate the presence of the *apxIV* gene in the various *A. pleuropneumoniae* serotypes and related bacteria, three probes were made (see fig. 4). Probe APXIVA is described in example 1. Probe APXIVA2 contains the 2015 bp DNA fragment between the *Bam*HI and *Nru*I sites. The 758 bp probe APPIVA1 was made by PCR amplification with oligos APPIV1-L (5'-GGGACAGTGGCTCAATTAAG-3') and (APPIV1-R (5'-AGCTGTAAACTCCACCAACG-3'). All probes were labelled with Digoxigenin-11-dUTP (Boehringer Mannheim) according to the protocol of the manufacturer and hybridised with Southern blots containing *Cla*I digested chromosomal DNA of all *A. pleuropneumoniae* reference strains and the HV114 field strain, *Actinobacillus suis* (ATCC 15558), *Actinobacillus rossii* (ATCC 27072) and *Actinobacillus equuli* (ATCC 19392). All three probes react similarly (see fig. 7 for the results with the APXIVA2 probe). All *A. pleuropneumoniae* strains react, whereas no hybridisation is observed with the *A. suis*, *A. equuli* and *A. rossii* strains.

Example 7:

Presence of *apxIV* genes in *A. pleuropneumoniae* and related strains using PCR amplification

With 50 ng of chromosomal DNA from the various *A. pleuropneumoniae* serotypes, other *Actinobacillus* species and *P. haemolytica* as templates, and primers APXIVA-1L (5'-TGGCACTGACGGTGATGA-3') and APXIVA-1R (5'-GGCCATCGACTCAACCAT-3') PCR

amplification was performed. After analysis of the products on an agarose gel, products with the expected size of 442 bp were observed in all *A. pleuropneumoniae* samples, but in none of the other *Actinobacillus* species (fig. 8). This indicates that in addition to the results in example 6, also PCR could be used to discriminate *A. pleuropneumoniae* from other *Actinobacillus* species.

Example 8:

Overexpression of ApxIV-var1 polyhistidine fusion protein.

Starting with plasmid pROK-7 (see example 1) as a template and oligonucleotides APX4/II5-L (5'-CGCCATATGACAAAATTA¹⁴CTATGCAAG) and APX4/II6-R (5'-CGCGAATTCAGCGACACAAGAGATAT¹⁵) as PCR-primers, a PCR fragment was amplified. A sufficient amount of this fragment was then digested with restriction enzymes NdeI and EcoRI and cloned in expression vector pETHIS-1, digested with the same enzymes as described in Example 3. From the resulting plasmid, designated pJFFApxIVA1His1, a 206 kD polyhistidine fusion protein (MW determined in PAGE) of 1841 amino acid residues was overexpressed in *E. coli* as described in Example 3. The protein is encoded in the coding region spanning nucleic acid no. 1132 to 6546 as depicted in SEQ ID NO: 5. The amino acid sequence of the protein is given in SEQ ID NO: 6. In Western blot this product was shown to react with specific anti-ApxIV serum 522-409 (antiserum described in example 3).

Example 9:

Protection of mice by vaccination with ApxIV against APP challenge.

The 206 kD polyhistidine-ApxIV fusion protein as described in Example 8 and a comparable 108 kD polyhistidine-ApxIA-fusion protein, both derived from serotype 1 reference strain 4074 genomic material, were overexpressed as described in example 3. The cell pellet of induced *E. coli* cells was resuspended into PBS buffer (1 g. cell pellet in 6 ml buffer) and sonicated on ice for two times 45 seconds on ice for lysis of the cells. After centrifugation for 20 minutes at 22.000 x g at 4°C, the supernatant was discarded and the resulting pellet was washed with a solution of 3M urea (pH 6.3). The urea was removed after centrifugation for 20 minutes at 22.000 x g, and the resulting pellet was solubilized in 6M GuanidiniumHCl (pH 8.0). The protein samples were standardised by specific protein content after densitometry of PAGE gels. The samples were diluted with PBS and formulated with an oil adjuvant.

Three groups of 15 mice each, were intraperitoneally immunised with the ApxIV antigen (36.3 microgram), ApxIA antigen (36.3 microgram), or the adjuvant alone. The vaccines were administered in a volume of 0.4 ml. Twenty-four days after the first vaccination, each group of mice was split in two groups of 7 and 8 mice which were boosted with half the amount of antigen. The groups of 7 mice were boosted intraperitoneally in a volume of 0.2 ml and the groups of 8 mice were vaccinated intramuscularly with 0.1 ml in each hind leg. Thirteen days after the booster, the mice were challenged intraperitoneally with 1.5×10^8 cfu of a virulent serotype 1 strain .

Example 10:

Poreforming capacity of ApxIV:

Freshly induced *E. coli* cells expressing ApxIV were used as the source of protein for testing poreformation in artificial lipid bilayers as described by Maier et al., *Infect. Immun.*, 64; 4415-4423(1996). The methods used for black lipid bilayer experiments have been described previously (Benz et al.; *Biochim. Biophys. Acta* 511: 305-319 (1978)). Membranes were formed from a 1% solution of asolectin (soybean lecitin type IV-S from Sigma, St. Louis MO) in n-decane. Zero current membrane potentials were measured with a Keithley 610 C electrometer 5-10 min. after a 10-fold salt gradient was established across the membranes (Benz et al.; *Biochim. Biophys. Acta* 551: 238-247 (1979)). The presence of ApxIV resulted in a high frequency of pore formation in the presence of 0.5 % cholesterol, with an average single channel conductance (G) of 4 nS.

These results indicate that the ApxIV induces pores into artificial bilayers and is therefore toxic to eukaryotic cells and thus is a virulence factor for *A. pleuropneumoniae*.

Legend to the figures:

Figure 1: Comparison of ApxIV^Avar1 and ApxIV^Avar3 (var stands for serotype). Graphic representation of the different features found in the C-terminal end. Dashed boxes represent the direct repeat regions in ApxIV^A. Bold vertical bars indicate the position of glycine rich nonapeptides, and DNA polymerase family B signatures are indicated by black triangles. The amino acid sequence YSDSANSKK represents the spacer sequence in the ApxIV^Avar3 gene. The sequence segment of ApxIV^Avar1 which is deleted in ApxIV^Avar3 is also indicated in the figure.

figure 2: Alignment of the amino acid sequences of direct repeat 1 (A), direct repeat 2 (B) and direct repeat 3 (C) of ApxIV^Avar1 and ApxIV^Avar3. The copies of each of the direct repeats 1, 2 and 3 are labeled by letters to distinguish them for the sequence comparison. Variant residues are shown in bold letters.

Figure 3: Partial restriction maps of pBLac7 (Thesis of T.J. Anderson University of Guelph, 1995), pROK7 and pROK5. The different open reading frames (ORF's) are indicated by arrows. The interrupted arrow of *lacZ* in pROK7 indicates partial sequencing of the gene. Potential *rho*-independent transcription terminators are indicated (W). Potential transcription start sites are indicated by a triangle. Restriction sites: A= *Asp700*; B= *BamHI*; C= *ClaI*; E= *EcoRV*; Ec= *EcoRI*; H= *HindIII*; N= *NruI*; Nd= *NdeI*; Nh= *NheI*; P= *PstI*; S= *SpeI*; Sm= *SmaI*.

Figure 4: Location of the various oligonucleotides and probes on the map of the *apxIV^Avar3* gene.

Figure 5: Expression of ApxIV in *in vitro* cultivated serotype 1 reference strain 4074. Panel A was reacted with the anti-ApxIA monoclonal antibody, panel B with anti-ApxIIA monoclonal antibody and panel C was reacted with anti-ApxIVA serum 522-409. Lane 1 contains ApxIA-polyhistidine fusion protein, lane 2 contains ApxIIA-polyhistidine fusion protein, lane 3 contains strain 4074 concentrated culture supernatant, lane 4 contains ApxIVA polyhistidine fusion protein.

Figure 6: Immunoblot showing the reactivities of sera from pigs which were experimentally infected with the reference strain of serotype 1 (lane 1) or pig sera from serotype 1 field infections (lane 2-4) with the 80 kD polyhistidine-ApxIV fusion protein. As a positive control (+), serum 522-409 was used, as a negative control (-), polyclonal rabbit serum against ApxI and ApxII (Frey et al., Infect. Immun., 57;2050-2056, 1989) was used as a 1000-fold dilution.

Figure 7: Southern blot of *Clal* digested genomic DNA hybridised with probe APXIVA2. Lanes 1-13: *A. pleuropneumoniae* reference strains; 1: serotype 1; 2: serotype 2; 3: serotype 3; 4: serotype 4; 5: serotype 5a; 6: serotype 5b; 7: serotype 6; 8: serotype 7; 9: serotype 8; 10: serotype 9; 11: serotype 10; 12: serotype 11; 13: serotype 12; 14: HV114 field strain; 15: *A. suis* (ATCC 15558); 16: *A. rossii* (ATCC 27072); 17: *A. equuli* (ATCC 19392). Molecular size markers are indicated (in kilobasepairs) on the left.

Figure 8: PCR amplification of *apxIV* using primers APXIVA-1L and APXIVA-1R. Lane assignments: lanes 1 to 13 contain the *A. pleuropneumoniae* reference strains from serotypes 1, 2, 3, 4, 5a, 5b, 6, 7, 8, 9, 10, 11 and 12 respectively; lane 14: strain HV114; lane 15: *A. suis* ATCC 15558; lane 16: *A. rossii* ATCC 27072; lane 17: *A. equuli* ATCC 19392; lane 18: *A. lignieresii* ATCC 49236; lane 19: *P. haemolytica* type 1 ATCC 14003. Molecular size markers (in kilobasepairs) are indicated on the left.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: AKZO Nobel N.V.
- (B) STREET: Velperweg 76
- (C) CITY: Arnhem
- (E) COUNTRY: The Netherlands
- (F) POSTAL CODE (ZIP): 6824 BM

(ii) TITLE OF INVENTION: Live attenuated Actinobacillus pleuropneumoniae

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6736 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Actinobacillus pleuropneumoniae
- (B) STRAIN: 4074 (serotype 1 reference strain)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: pROK7

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1576..6549
- (D) OTHER INFORMATION: /codon_start= 1576
/function= "RTX toxin"
/product= "ApxIV_var1"
/gene= "apxIV_var1"
/number= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CATATCTGCC AAAATTGCGG TCACCACGAA GATATTTTCG GCACCGGCGG TGCGGAGAAA      240
GTGGCGAAGA AATACGGTAC TAAAGTATTA GGACAAATGC CGTTGCATAT TCGCTTACGT      300
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95 100 105	

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AAA Lys 125	CCG Pro 125	ATT Ile 125	ATT Ile 125	TAC Tyr 130	GGC Gly 130	ACG Thr 130	GCC Ala 130	GGC Gly 130	AAC Asn 135	GAC Asp 135	AGT Ser 135	AAG Lys 135	ATT Ile 135	GAA Glu 140	GGC Gly 140	1995
ACT Thr 145	AAA Lys 145	ATC Ile 145	ACC Thr 145	CGT Arg 145	AGG Arg 145	ATT Ile 145	GCG Ala 150	GGT Gly 150	AAA Lys 150	GAA Glu 150	GTT Val 150	ACG Thr 155	CTT Leu 155	GAT Asp 155	ATT Ile 155	2043
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GTT Val 175	AGT Ser 175	GGT Gly 175	TCG Ser 175	GAT Asp 175	ATC Ile 180	ATT Ile 180	AAA Lys 180	TTG Leu 180	TTG Leu 180	TTT Phe 185	GGA Gly 185	GCA Ala 185	TTG Leu 185	ACT Thr 185	CCA Pro 185	2139
ACT Thr 190	TTA Leu 190	AAT Asn 190	AGA Arg 190	ATG Met 195	TTG Leu 195	CTA Leu 195	TCA Ser 195	CAA Gln 195	CTT Leu 195	ATC Ile 200	CAG Gln 200	TCT Ser 200	TTT Phe 200	TCC Ser 200	GAT Asp 200	2187
AGC Ser 205	TTG Leu 205	GCT Ala 205	AAA Lys 205	CTT Leu 210	GAT Asp 210	AAT Asn 210	CCC Pro 210	TTA Leu 210	GCC Ala 215	CCT Pro 215	TAC Tyr 215	ACT Thr 215	AAA Lys 215	AAT Asn 220	GGC Gly 220	2235
GTG Val 225	GTT Val 225	TAT Tyr 225	GTC Val 225	ACC Thr 225	GGC Gly 225	AAA Lys 225	GGG Gly 230	AAT Asn 230	GAT Asp 230	GTG Val 230	CTT Leu 230	AAA Lys 230	GGA Gly 235	ACT Thr 235	GAA Glu 235	2283
CAT His 240	GAG Glu 240	GAT Asp 240	TTG Leu 240	TTT Phe 240	CTC Leu 240	GGT Gly 245	GGT Gly 245	GAG Glu 245	GGG Gly 245	AAT Asn 245	GAT Asp 245	ACT Thr 250	TAT Tyr 250	TAT Tyr 250	GCG Ala 250	2331
AGA Arg 255	GTA Val 255	GGC Gly 255	GAT Asp 255	ACA Thr 255	ATT Ile 255	GAA Glu 260	GAC Asp 260	GCC Ala 260	GAC Asp 260	GGC Gly 265	AAA Lys 265	GGT Gly 265	AAA Lys 265	GTC Val 265	TAT Tyr 265	2379
TTT Phe 270	GTG Val 270	AGA Arg 270	GAA Glu 270	AAA Lys 270	GGG Gly 275	GTA Val 275	CCT Pro 275	AAG Lys 275	GCG Ala 280	GAT Asp 280	CCT Pro 280	AAG Lys 280	CGG Arg 280	GTA Val 280	GAG Glu 280	2427
TTT Phe 285	AGC Ser 285	GAG Glu 285	TAC Tyr 285	ATA Ile 285	ACG Thr 290	AAA Lys 290	GAA Glu 290	GAA Glu 290	ATA Ile 295	AAA Lys 295	GAG Glu 295	GTT Val 295	GAA Glu 295	AAG Lys 295	GGG Gly 300	2475
TTA Leu 305	TTA Leu 305	ACT Thr 305	TAC Tyr 305	GCA Ala 305	GTT Val 305	TTA Leu 310	GAA Glu 310	AAT Asn 310	TAT Tyr 310	AAT Asn 310	TGG Trp 310	GAA Glu 315	GAG Glu 315	AAA Lys 315	ACG Thr 315	2523
GCG Ala 320	ACT Thr 320	TTC Phe 320	GCT Ala 320	CAT His 320	GCG Ala 325	ACT Thr 325	ATG Met 325	CTT Leu 325	AAT Asn 325	GAG Glu 330	CTT Leu 330	TTT Phe 330	ACT Thr 330	GAT Asp 330	TAT Tyr 330	2571
ACT Thr 335	AAT Asn 335	TAT Tyr 335	CGT Arg 335	TAT Tyr 335	GAA Glu 340	GTT Val 340	AAA Lys 340	GGA Gly 340	CTA Leu 345	AAA Lys 345	TTG Leu 345	CCC Pro 345	GCC Ala 345	GTT Val 345	AAA Lys 345	2619
AAG Lys 350	TTA Leu 350	AAA Lys 350	AGT Ser 350	CCG Pro 350	TTG Leu 355	GTG Val 355	GAG Glu 355	TTT Phe 355	ACA Thr 360	GCT Ala 360	GAT Asp 360	TTA Leu 360	TTA Leu 360	ACT Thr 360	GTT Val 360	2667
ACG Thr 365	CCT Pro 365	ATT Ile 365	GAC Asp 365	GAA Glu 370	AAC Asn 370	GGA Gly 370	AAA Lys 370	GCA Ala 375	CTT Leu 375	AGC Ser 375	GAA Glu 375	AAA Lys 375	AGT Ser 375	ATT Ile 375	ACG Thr 380	2715

GTT AAA AAT TTT AAA AAT GGT GAT TTA GGA ATA AGG TTG TTG GAT CCT Val Lys Asn Phe Lys Asn Gly Asp Leu Gly Ile Arg Leu Leu Asp Pro 385 390 395	2763
AAT AGC TAT TAT TAT TTC CTT GAA GGC CAA GAT ACG GGT TTT TAT GGT Asn Ser Tyr Tyr Tyr Phe Leu Glu Gly Gln Asp Thr Gly Phe Tyr Gly 400 405 410	2811
CCT GCT TTT TAT ATT GAA CGA AAA AAC GGT GGC GGC GCT AAA AAT AAC Pro Ala Phe Tyr Ile Glu Arg Lys Asn Gly Gly Gly Ala Lys Asn Asn 415 420 425	2859
TCG TCG GGA GCA GGA AAT AGC AAA GAT TGG GGC GGG AAC GGG CAT GGA Ser Ser Gly Ala Gly Asn Ser Lys Asp Trp Gly Asn Gly His Gly 430 435 440	2907
AAT CAC CGA AAT AAT GCC TCC GAC CTG AAT AAA CCG GAC GGA AAT AAT Asn His Arg Asn Asn Ala Ser Asp Leu Asn Lys Pro Asp Gly Asn Asn 445 450 455 460	2955
GGG AAT AAC CAA AAT AAC GGA AGC AAT CAA GAT AAT CAT AGC GAT GTG Gly Asn Asn Gln Asn Asn Gly Ser Asn Gln Asp Asn His Ser Asp Val 465 470 475	3003
AAT GCG CCA AAT AAC CCG GGA CGT AAC TAT GAT ATT TAC GAT CCT TTA Asn Ala Pro Asn Asn Pro Gly Arg Asn Tyr Asp Ile Tyr Asp Pro Leu 480 485 490	3051
GCT TTA GAT TTA GAT GGA GAT GGG CTT GAA ACC GTG TCG ATG AAC GGG Ala Leu Asp Leu Asp Gly Asp Gly Leu Glu Thr Val Ser Met Asn Gly 495 500 505	3099
CGA CAA GGC GCG TTA TTC GAT CAT GAA GGA AAA GGT ATT CGT ACC GCA Arg Gln Gly Ala Leu Phe Asp His Glu Gly Lys Gly Ile Arg Thr Ala 510 515 520	3147
ACG GGC TGG CTC GCT GCG GAT GAC GGT TTT TTA GTG TTA GAT CGT AAC Thr Gly Trp Leu Ala Ala Asp Asp Gly Phe Leu Val Leu Asp Arg Asn 525 530 535 540	3195
CAA GAC GGC ATT ATT AAT GAT ATA AGC GAG TTA TTT AGT AAT AAA AAT Gln Asp Gly Ile Ile Asn Asp Ile Ser Glu Leu Phe Ser Asn Lys Asn 545 550 555	3243
CAA CTT TCC GAC GGC AGT ATT TCT GCA CAC GGT TTT GCG ACA TTA GCC Gln Leu Ser Asp Gly Ser Ile Ser Ala His Gly Phe Ala Thr Leu Ala 560 565 570	3291
GAT TTG GAT ACA AAC CAA GAT CAG CGT ATC GAC CAA AAT GAT AAG CTG Asp Leu Asp Thr Asn Gln Asp Gln Arg Ile Asp Gln Asn Asp Lys Leu 575 580 585	3339
TTT TCT AAA CTC CAA ATT TGG CGG GAT TTA AAT CAA AAC GGT TTT AGT Phe Ser Lys Leu Gln Ile Trp Arg Asp Leu Asn Gln Asn Gly Phe Ser 590 595 600	3387
GAA GCG AAT GAG CTG TTT AGC TTA GAA AGT TTG AAT ATT AAA TCT TTA Glu Ala Asn Glu Leu Phe Ser Leu Glu Ser Leu Asn Ile Lys Ser Leu 605 610 615 620	3435
CAT ACC GCC TAT GAA GAG CGT AAT GAT TTT CTA GCG GGC AAT AAT ATC His Thr Ala Tyr Glu Glu Arg Asn Asp Phe Leu Ala Gly Asn Asn Ile 625 630 635	3483
CTT GCT CAG CTT GGG AAG TAT GAA AAA ACG GAC GGT ACT TTT GCA CAA Leu Ala Gln Leu Gly Lys Tyr Glu Lys Thr Asp Gly Thr Phe Ala Gln 640 645 650	3531

ATG GGC GAT TTA AAT TTC AGT TTT AAC CCG TTT TAT AGC CGA TTT ACC Met Gly Asp Leu Asn Phe Ser Phe Asn Pro Phe Tyr Ser Arg Phe Thr 655 660 665	3579
GAA GCG TTA AAT TTA ACC GAG CAA CAA CGT CGC ACA ATT AAT CTA ACC Glu Ala Leu Asn Leu Thr Glu Gln Gln Arg Arg Thr Ile Asn Leu Thr 670 675 680	3627
GGC ACC GGT CGG GTT CGG GAT TTG CGT GAA GCC GCC GCA CTT TCT GAG Gly Thr Gly Arg Val Arg Asp Leu Arg Glu Ala Ala Ala Leu Ser Glu 685 690 695 700	3675
GAG TTG GCT GCT TTA TTA CAA CAG TAC ACT AAG GCC TCC GAT TTT CAG Glu Leu Ala Ala Leu Leu Gln Gln Tyr Thr Lys Ala Ser Asp Phe Gln 705 710 715	3723
GCA CAA CGA GAA TTA TTG CCT GCC ATT TTA GAT AAA TGG GCG GCA ACG Ala Gln Arg Glu Leu Leu Pro Ala Ile Leu Asp Lys Trp Ala Ala Thr 720 725 730	3771
GAT TTA CAG TAT CAA CAT TAT GAT AAA ACA TTA CTT AAA ACG GTA GAA Asp Leu Gln Tyr Gln His Tyr Asp Lys Thr Leu Leu Lys Thr Val Glu 735 740 745	3819
AGT ACC GAT AGT AGT GCT TCT GTC GTT AGA GTC ACG CCT TCT CAA TTA Ser Thr Asp Ser Ser Ala Ser Val Val Arg Val Thr Pro Ser Gln Leu 750 755 760	3867
AGT AGT ATA CGC AAT GCA AAG CAT GAT CCT ACC GTT ATG CAA AAC TTT Ser Ser Ile Arg Asn Ala Lys His Asp Pro Thr Val Met Gln Asn Phe 765 770 775 780	3915
GAA CAG AGT AAG GCA AAA ATT GCG ACT TTA AAT TCG CTC TAC GGG TTA Glu Gln Ser Lys Ala Lys Ile Ala Thr Leu Asn Ser Leu Tyr Gly Leu 785 790 795	3963
AAT ATC GAT CAA CTT TAT TAC ACG ACG GAT AAA GAC ATT CGC TAT ATT Asn Ile Asp Gln Leu Tyr Tyr Thr Thr Asp Lys Asp Ile Arg Tyr Ile 800 805 810	4011
ACT GAT AAA GTG AAT AAT ATG TAT CAA ACA ACC GTA GAA CTT GCC TAC Thr Asp Lys Val Asn Asn Met Tyr Gln Thr Thr Val Glu Leu Ala Tyr 815 820 825	4059
CGT TCT TTA CTT TTA CAA ACG CGT TTG AAG AAA TAT GTT TAT AGC GTT Arg Ser Leu Leu Leu Gln Thr Arg Leu Lys Lys Tyr Val Tyr Ser Val 830 835 840	4107
AAT GCG AAA CAA TTC GAA GGG AAA TGG GTA ACC GAT TAT TCT CGT ACT Asn Ala Lys Gln Phe Glu Gly Lys Trp Val Thr Asp Tyr Ser Arg Thr 845 850 855 860	4155
GAA GCC TTA TTT AAC TCT ACT TTT AAA CAA TCG CCT GAA AAT GCA TTA Glu Ala Leu Phe Asn Ser Thr Phe Lys Gln Ser Pro Glu Asn Ala Leu 865 870 875	4203
TAT GAT TTA AGC GAA TAC CTT TCT TTC TTT AAC GAT CCT ACG GAA TGG Tyr Asp Leu Ser Glu Tyr Leu Ser Phe Phe Asn Asp Pro Thr Glu Trp 880 885 890	4251
AAA GAA GGG CTA TTA CTG TTA AGC CGT TAT ATA GAT TAT GCT AAA GCA Lys Glu Gly Leu Leu Leu Leu Ser Arg Tyr Ile Asp Tyr Ala Lys Ala 895 900 905	4299
CAA GGA TTT TAT GAA AAC TGG GCG GCT ACT TCT AAC TTA ACT ATT GCC Gln Gly Phe Tyr Glu Asn Trp Ala Ala Thr Ser Asn Leu Thr Ile Ala 910 915 920	4347

CGT	TTA	AGA	GAG	GCT	GGA	GTA	ATT	TTT	GCA	GAA	TCG	ACG	GAT	TTA	AAA	4395
Arg	Leu	Arg	Glu	Ala	Gly	Val	Ile	Phe	Ala	Glu	Ser	Thr	Asp	Leu	Lys	
925					930					935					940	
GGC	GAT	GAA	AAA	AAT	AAT	ATT	TTG	TTA	GGT	AGC	CAA	AAA	GAT	AAT	AAC	4443
Gly	Asp	Glu	Lys	Asn	Asn	Ile	Leu	Leu	Gly	Ser	Gln	Lys	Asp	Asn	Asn	
				945					950					955		
TTA	TCG	GGT	AGT	GCA	GGT	GAT	GAT	CTA	CTT	ATC	GGC	GGA	GAG	GGT	AAT	4491
Leu	Ser	Gly	Ser	Ala	Gly	Asp	Asp	Leu	Leu	Ile	Gly	Gly	Glu	Gly	Asn	
			960					965					970			
GAT	ACG	TTA	AAA	GGC	AGC	TAC	GGT	GCA	GAC	ACC	TAT	ATC	TTT	AGC	AAA	4539
Asp	Thr	Leu	Lys	Gly	Ser	Tyr	Gly	Ala	Asp	Thr	Tyr	Ile	Phe	Ser	Lys	
		975					980					985				
GGA	CAC	GGA	CAG	GAT	ATC	GTT	TAT	GAA	GAT	ACC	AAT	AAT	GAT	AAC	CGC	4587
Gly	His	Gly	Gln	Asp	Ile	Val	Tyr	Glu	Asp	Thr	Asn	Asn	Asp	Asn	Arg	
	990					995					1000					
GCA	AGA	GAT	ATC	GAC	ACC	TTA	AAA	TTT	ACC	GAT	GTG	AAT	TAT	GCG	GAA	4635
Ala	Arg	Asp	Ile	Asp	Thr	Leu	Lys	Phe	Thr	Asp	Val	Asn	Tyr	Ala	Glu	
1005						1010				1015					1020	
GTG	AAG	TTT	CGA	CGA	GTA	GAT	AAT	GAC	TTA	ATG	TTA	TTC	GGT	TAT	CAT	4683
Val	Lys	Phe	Arg	Arg	Val	Asp	Asn	Asp	Leu	Met	Leu	Phe	Gly	Tyr	His	
				1025					1030					1035		
GAT	ACG	GAT	TCG	GTC	ACG	GTA	AAA	TCC	TTC	TAC	AGC	CAT	GTA	GAT	TAT	4731
Asp	Thr	Asp	Ser	Val	Thr	Val	Lys	Ser	Phe	Tyr	Ser	His	Val	Asp	Tyr	
			1040					1045					1050			
CAA	TTT	GAC	AAA	TTG	GAG	TTT	GCT	GAC	CGC	AGT	ATA	ACT	CGC	GAT	GAA	4779
Gln	Phe	Asp	Lys	Leu	Glu	Phe	Ala	Asp	Arg	Ser	Ile	Thr	Arg	Asp	Glu	
		1055					1060					1065				
CTG	ATT	AAA	GCA	GGG	CTT	CAT	CTA	TAC	GGC	ACC	GAT	GGC	AAT	GAT	GAT	4827
Leu	Ile	Lys	Ala	Gly	Leu	His	Leu	Tyr	Gly	Thr	Asp	Gly	Asn	Asp	Asp	
	1070					1075					1080					
ATA	AAG	GAT	CAT	GCG	GAT	TGG	GAC	AGC	ATT	TTG	GAA	GGC	GGC	AAA	GGC	4875
Ile	Lys	Asp	His	Ala	Asp	Trp	Asp	Ser	Ile	Leu	Glu	Gly	Gly	Lys	Gly	
1085					1090					1095					1100	
AAC	GAT	ATT	CTA	AGA	GGT	GGC	TAC	GGT	GCG	GAC	ACC	TAT	ATC	TTT	AGC	4923
Asn	Asp	Ile	Leu	Arg	Gly	Gly	Tyr	Gly	Ala	Asp	Thr	Tyr	Ile	Phe	Ser	
			1105						1110					1115		
AAA	GGA	CAC	GGA	CAG	GAT	ATC	GTT	TAT	GAA	GAT	ACC	AAT	AAT	GAT	AAC	4971
Lys	Gly	His	Gly	Gln	Asp	Ile	Val	Tyr	Glu	Asp	Thr	Asn	Asn	Asp	Asn	
			1120					1125					1130			
CGC	GCA	AGA	GAT	ATC	GAC	ACC	TTA	AAA	TTT	ACT	GAT	GTG	AAT	TAT	GCG	5019
Arg	Ala	Arg	Asp	Ile	Asp	Thr	Leu	Lys	Phe	Thr	Asp	Val	Asn	Tyr	Ala	
		1135					1140					1145				
GAA	GTG	AAA	TTC	CGA	CGA	GTA	GAT	AAT	GAC	TTA	ATG	TTA	TTC	GGT	TAT	5067
Glu	Val	Lys	Phe	Arg	Arg	Val	Asp	Asn	Asp	Leu	Met	Leu	Phe	Gly	Tyr	
	1150					1155					1160					
CAT	GAT	ACG	GAT	TCG	GTC	ACG	ATA	AAA	TCC	TTC	TAC	AAC	CAT	GTA	GAT	5115
His	Asp	Thr	Asp	Ser	Val	Thr	Ile	Lys	Ser	Phe	Tyr	Asn	His	Val	Asp	
1165					1170					1175				1180		
TAT	CAA	TTT	GAC	AAA	TTG	GAA	TTT	GCT	GAC	CGC	AGT	ATA	ACT	CGT	GAT	5163
Tyr	Gln	Phe	Asp	Lys	Leu	Glu	Phe	Ala	Asp	Arg	Ser	Ile	Thr	Arg	Asp	
				1185					1190					1195		

GAA CTA GGT AAA CAA GGT ATG GCA TTA TTT GGC ACT GAC GGT GAT GAT	5211
Glu Leu Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp	
1200 1205 1210	
AAT ATC AAC GAC TGG GGA CGT AAC TCG GTG ATT GAT GCC GGT GCG GGT	5259
Asn Ile Asn Asp Trp Gly Arg Asn Ser Val Ile Asp Ala Gly Ala Gly	
1215 1220 1225	
AAT GAT ACG GTT AAT GGC GGT AAT GGC GAT GAC ACC CTC ATC GGC GGC	5307
Asn Asp Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly	
1230 1235 1240	
AAA GGT AAT GAT ATT CTA AGA GGT GGC TAC GGT GCG GAC ACC TAT ATC	5355
Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile	
1245 1250 1255 1260	
TTT AGC AAA GGA CAC GGA CAG GAT ATC GTT TAT GAA GAT ACC AAT AAT	5403
Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn	
1265 1270 1275	
GAT AAC CGC GCA AGA GAT ATC GAC ACC TTA AAA TTT ACC GAT GTG AAT	5451
Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn	
1280 1285 1290	
TAT GCG GAA GTG AAA TTC CGA CGA GTA GAT AAT GAC TTA ATG TTA TTC	5499
Tyr Ala Glu Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe	
1295 1300 1305	
GGT TAT CAT GAT ACG GAT TCG GTC ACG GTA AAA TCC TTC TAC AGC CAT	5547
Gly Tyr His Asp Thr Asp Ser Val Thr Val Lys Ser Phe Tyr Ser His	
1310 1315 1320	
GTA GAT TAT CAA TTT GAC AAA TTG GAG TTT GCT GAC CGC AGT ATA ACT	5595
Val Asp Tyr Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr	
1325 1330 1335 1340	
CGC GAT GAA CTG ATT AAA GCA GGG CTT CAT CTA TAC GGC ACC GAT GGC	5643
Arg Asp Glu Leu Ile Lys Ala Gly Leu His Leu Tyr Gly Thr Asp Gly	
1345 1350 1355	
AAT GAT GAT ATA AAG GAT CAT GCG GAT TGG GAC AGC ATT TTG GAA GGC	5691
Asn Asp Asp Ile Lys Asp His Ala Asp Trp Asp Ser Ile Leu Glu Gly	
1360 1365 1370	
GGC AAA GGC AAC GAT ATT CTA AGA GGT GGC TAC GGT GCG GAC ACC TAT	5739
Gly Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr	
1375 1380 1385	
ATC TTT AGC AAA GGA CAC GGA CAG GAT ATC GTT TAT GAA GAT ACC AAT	5787
Ile Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn	
1390 1395 1400	
AAT GAT AAC CGA GCA AGA GAT ATC GAC ACC TTA AAA TTT ACT GAT GTG	5835
Asn Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val	
1405 1410 1415 1420	
AAT TAT GCG GAA GTG AAA TTC CGA CGA GTA GAT AAT GAC TTA ATG TTA	5883
Asn Tyr Ala Glu Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu	
1425 1430 1435	
TTC GGT TAT CAT GAT ACG GAT TCG GTC ACG ATA AAA TCC TTC TAC AAC	5931
Phe Gly Tyr His Asp Thr Asp Ser Val Thr Ile Lys Ser Phe Tyr Asn	
1440 1445 1450	
CAT GTA GAT TAT CAA TTT GAC AAA TTG GAA TTT GCT GAC CGC AGT ATA	5979
His Val Asp Tyr Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile	
1455 1460 1465	

ACT CGT GAT GAA CTA GGT AAA CAA GGT ATG GCA TTA TTT GGC ACT GAC Thr Arg Asp Glu Leu Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp 1470 1475 1480	6027
GGT GAT GAT AAT ATC AAC GAC TGG GGA CGT AAC TCG GTG ATT GAT GCC Gly Asp Asp Asn Ile Asn Asp Trp Gly Arg Asn Ser Val Ile Asp Ala 1485 1490 1495 1500	6075
GGT GCG GGT AAT GAT ACG GTT AAT GGC GGT AAT GGC GAT GAC ACC CTC Gly Ala Gly Asn Asp Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu 1505 1510 1515	6123
ATC GGC GGC AAA GGT AAT GAT ATT CTA AGA GGT GGC TAC GGT GCG GAC Ile Gly Gly Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp 1520 1525 1530	6171
ACC TAT ATC TTT AGC AAA GGA CAC GGA CAG GAT ATC GTT TAT GAA GAT Thr Tyr Ile Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp 1535 1540 1545	6219
ACC AAT AAT GAT AAC CGC GCA AGA GAT ATC GAC ACC TTA AAA TTT ACT Thr Asn Asn Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr 1550 1555 1560	6267
GAT ATT AAT TTA TCC GAA CTT TGG TTT AGC CGA GAA AAT AAC GAT TTG Asp Ile Asn Leu Ser Glu Leu Trp Phe Ser Arg Glu Asn Asn Asp Leu 1565 1570 1575 1580	6315
ATT ATT AAA TCA TTA TTA AGT GAG GAT AAA GTC ACG GTT CAA AAT TGG Ile Ile Lys Ser Leu Leu Ser Glu Asp Lys Val Thr Val Gln Asn Trp 1585 1590 1595	6363
TAT TCA CAC CAA GAT CAT AAA ATA GAA AAT ATT CGT TTA TCG AAT GAG Tyr Ser His Gln Asp His Lys Ile Glu Asn Ile Arg Leu Ser Asn Glu 1600 1605 1610	6411
CAA ACG TTG GTG AGC ACT CAG GTG GAG AAG ATG GTT GAG TCG ATG GCC Gln Thr Leu Val Ser Thr Gln Val Glu Lys Met Val Glu Ser Met Ala 1615 1620 1625	6459
GGC TTT GCT CAG AAG CAC GGA GGA GAG ATA TCT CTT GTG TCG CTT GAA Gly Phe Ala Gln Lys His Gly Gly Glu Ile Ser Leu Val Ser Leu Glu 1630 1635 1640	6507
GAG GTA AAA CAA TAT ATC AAT AGC TTA ACA GCT GCT TTA TAA Glu Val Lys Gln Tyr Ile Asn Ser Leu Thr Ala Ala Leu *	6549
1645 1650 1655	
CATACGAAAG AAATCGGCAC AGTTTTTTTTG AACTGTGCCG ATTTGATTTT AGTGTAAGAA	6609
TATAGCCTGA TTTTAAGAAA TTTACTCTTG GCTAATAACT ATTTCCCATTTT TTATAAGTTA	6669
TTGACGGATG GTTTTATCAA ATATGAGATC AAATCTTATT TTAAATTTCG TTTCCATTAA	6729
GCGATAT	6736

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1658 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Ser Asp Asn Ala Phe Phe Val Ile Glu Glu Ser Gly Lys Arg Tyr
 1           5           10           15
Ile Glu Asn Phe Gly Ile Glu Pro Leu Gly Lys Gln Glu Asp Phe Asp
          20           25           30
Phe Val Gly Gly Phe Trp Ser Asn Leu Val Asn Arg Gly Leu Glu Ser
          35           40           45
Ile Ile Asp Pro Ser Gly Ile Gly Gly Thr Val Asn Leu Asn Phe Thr
          50           55           60
Gly Glu Val Glu Thr Tyr Thr Leu Asp Glu Thr Arg Phe Lys Ala Glu
          65           70           75           80
Ala Ala Lys Lys Ser His Trp Ser Leu Val Asn Ala Ala Lys Val Tyr
          85           90           95
Gly Gly Leu Asp Gln Ile Ile Lys Lys Leu Trp Asp Ser Gly Ser Ile
          100          105          110
Lys His Leu Tyr Gln Asp Lys Asp Thr Gly Lys Leu Lys Pro Ile Ile
          115          120          125
Tyr Gly Thr Ala Gly Asn Asp Ser Lys Ile Glu Gly Thr Lys Ile Thr
          130          135          140
Arg Arg Ile Ala Gly Lys Glu Val Thr Leu Asp Ile Ala Asn Gln Lys
          145          150          155          160
Ile Glu Lys Gly Val Leu Glu Lys Leu Gly Leu Ser Val Ser Gly Ser
          165          170          175
Asp Ile Ile Lys Leu Leu Phe Gly Ala Leu Thr Pro Thr Leu Asn Arg
          180          185          190
Met Leu Leu Ser Gln Leu Ile Gln Ser Phe Ser Asp Ser Leu Ala Lys
          195          200          205
Leu Asp Asn Pro Leu Ala Pro Tyr Thr Lys Asn Gly Val Val Tyr Val
          210          215          220
Thr Gly Lys Gly Asn Asp Val Leu Lys Gly Thr Glu His Glu Asp Leu
          225          230          235          240
Phe Leu Gly Gly Glu Gly Asn Asp Thr Tyr Tyr Ala Arg Val Gly Asp
          245          250          255
Thr Ile Glu Asp Ala Asp Gly Lys Gly Lys Val Tyr Phe Val Arg Glu
          260          265          270
Lys Gly Val Pro Lys Ala Asp Pro Lys Arg Val Glu Phe Ser Glu Tyr
          275          280          285
Ile Thr Lys Glu Glu Ile Lys Glu Val Glu Lys Gly Leu Leu Thr Tyr
          290          295          300
Ala Val Leu Glu Asn Tyr Asn Trp Glu Glu Lys Thr Ala Thr Phe Ala
          305

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His Ala Thr Met Leu Asn Glu Leu Phe Thr Asp Tyr Thr Asn Tyr Arg
 325 330 335
 Tyr Glu Val Lys Gly Leu Lys Leu Pro Ala Val Lys Lys Leu Lys Ser
 340 345 350
 Pro Leu Val Glu Phe Thr Ala Asp Leu Leu Thr Val Thr Pro Ile Asp
 355 360 365
 Glu Asn Gly Lys Ala Leu Ser Glu Lys Ser Ile Thr Val Lys Asn Phe
 370 375 380
 Lys Asn Gly Asp Leu Gly Ile Arg Leu Leu Asp Pro Asn Ser Tyr Tyr
 385 390 395 400
 Tyr Phe Leu Glu Gly Gln Asp Thr Gly Phe Tyr Gly Pro Ala Phe Tyr
 405 410 415
 Ile Glu Arg Lys Asn Gly Gly Gly Ala Lys Asn Asn Ser Ser Gly Ala
 420 425 430
 Gly Asn Ser Lys Asp Trp Gly Gly Asn Gly His Gly Asn His Arg Asn
 435 440 445
 Asn Ala Ser Asp Leu Asn Lys Pro Asp Gly Asn Asn Gly Asn Asn Gln
 450 455 460
 Asn Asn Gly Ser Asn Gln Asp Asn His Ser Asp Val Asn Ala Pro Asn
 465 470 475 480
 Asn Pro Gly Arg Asn Tyr Asp Ile Tyr Asp Pro Leu Ala Leu Asp Leu
 485 490 495
 Asp Gly Asp Gly Leu Glu Thr Val Ser Met Asn Gly Arg Gln Gly Ala
 500 505 510
 Leu Phe Asp His Glu Gly Lys Gly Ile Arg Thr Ala Thr Gly Trp Leu
 515 520 525
 Ala Ala Asp Asp Gly Phe Leu Val Leu Asp Arg Asn Gln Asp Gly Ile
 530 535 540
 Ile Asn Asp Ile Ser Glu Leu Phe Ser Asn Lys Asn Gln Leu Ser Asp
 545 550 555 560
 Gly Ser Ile Ser Ala His Gly Phe Ala Thr Leu Ala Asp Leu Asp Thr
 565 570 575
 Asn Gln Asp Gln Arg Ile Asp Gln Asn Asp Lys Leu Phe Ser Lys Leu
 580 585 590
 Gln Ile Trp Arg Asp Leu Asn Gln Asn Gly Phe Ser Glu Ala Asn Glu
 595 600 605
 Leu Phe Ser Leu Glu Ser Leu Asn Ile Lys Ser Leu His Thr Ala Tyr
 610 615 620
 Glu Glu Arg Asn Asp Phe Leu Ala Gly Asn Asn Ile Leu Ala Gln Leu
 625 630 635 640
 Gly Lys Tyr Glu Lys Thr Asp Gly Thr Phe Ala Gln Met Gly Asp Leu
 645 650 655
 Asn Phe Ser Phe Asn Pro Phe Tyr Ser Arg Phe Thr Glu Ala Leu Asn
 660 665 670
 Leu Thr Glu Gln Gln Arg Arg Thr Ile Asn Leu Thr Gly Thr Gly Arg
 675 680 685

Val Arg Asp Leu Arg Glu Ala Ala Ala Leu Ser Glu Glu Leu Ala Ala
 690 695 700
 Leu Leu Gln Gln Tyr Thr Lys Ala Ser Asp Phe Gln Ala Gln Arg Glu
 705 710 715 720
 Leu Leu Pro Ala Ile Leu Asp Lys Trp Ala Ala Thr Asp Leu Gln Tyr
 725 730 735
 Gln His Tyr Asp Lys Thr Leu Leu Lys Thr Val Glu Ser Thr Asp Ser
 740 745 750
 Ser Ala Ser Val Val Arg Val Thr Pro Ser Gln Leu Ser Ser Ile Arg
 755 760 765
 Asn Ala Lys His Asp Pro Thr Val Met Gln Asn Phe Glu Gln Ser Lys
 770 775 780
 Ala Lys Ile Ala Thr Leu Asn Ser Leu Tyr Gly Leu Asn Ile Asp Gln
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 Leu Tyr Tyr Thr Thr Asp Lys Asp Ile Arg Tyr Ile Thr Asp Lys Val
 805 810 815
 Asn Asn Met Tyr Gln Thr Thr Val Glu Leu Ala Tyr Arg Ser Leu Leu
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 Leu Gln Thr Arg Leu Lys Lys Tyr Val Tyr Ser Val Asn Ala Lys Gln
 835 840 845
 Phe Glu Gly Lys Trp Val Thr Asp Tyr Ser Arg Thr Glu Ala Leu Phe
 850 855 860
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 865 870 875 880
 Glu Tyr Leu Ser Phe Phe Asn Asp Pro Thr Glu Trp Lys Glu Gly Leu
 885 890 895
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 Glu Asn Trp Ala Ala Thr Ser Asn Leu Thr Ile Ala Arg Leu Arg Glu
 915 920 925
 Ala Gly Val Ile Phe Ala Glu Ser Thr Asp Leu Lys Gly Asp Glu Lys
 930 935 940
 Asn Asn Ile Leu Leu Gly Ser Gln Lys Asp Asn Asn Leu Ser Gly Ser
 945 950 955 960
 Ala Gly Asp Asp Leu Leu Ile Gly Gly Glu Gly Asn Asp Thr Leu Lys
 965 970 975
 Gly Ser Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly His Gly Gln
 980 985 990
 Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg Ala Arg Asp Ile
 995 1000 1005
 Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala Glu Val Lys Phe Arg
 1010 1015 1020
 Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His Asp Thr Asp Ser
 1025 1030 1035 1040
 Val Thr Val Lys Ser Phe Tyr Ser His Val Asp Tyr Gln Phe Asp Lys
 1045 1050 1055

Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu Ile Lys Ala
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 Gly Leu His Leu Tyr Gly Thr Asp Gly Asn Asp Asp Ile Lys Asp His
 1075 1080 1085
 Ala Asp Trp Asp Ser Ile Leu Glu Gly Gly Lys Gly Asn Asp Ile Leu
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 Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly His Gly
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 Ile Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala Glu Val Lys Phe
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 Ser Val Thr Ile Lys Ser Phe Tyr Asn His Val Asp Tyr Gln Phe Asp
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 Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu Gly Lys
 1185 1190 1195 1200
 Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp Asn Ile Asn Asp
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 Trp Gly Arg Asn Ser Val Ile Asp Ala Gly Ala Gly Asn Asp Thr Val
 1220 1225 1230
 Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly Lys Gly Asn Asp
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 Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly
 1250 1255 1260
 His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg Ala
 1265 1270 1275 1280
 Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala Glu Val
 1285 1290 1295
 Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His Asp
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 Thr Asp Ser Val Thr Val Lys Ser Phe Tyr Ser His Val Asp Tyr Gln
 1315 1320 1325
 Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu
 1330 1335 1340
 Ile Lys Ala Gly Leu His Leu Tyr Gly Thr Asp Gly Asn Asp Asp Ile
 1345 1350 1355 1360
 Lys Asp His Ala Asp Trp Asp Ser Ile Leu Glu Gly Gly Lys Gly Asn
 1365 1370 1375
 Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys
 1380 1385 1390
 Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg
 1395 1400 1405
 Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala Glu
 1410 1415 1420

Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His
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 Asp Thr Asp Ser Val Thr Ile Lys Ser Phe Tyr Asn His Val Asp Tyr
 1445 1450 1455
 Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu
 1460 1465 1470
 Leu Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp Asn
 1475 1480 1485
 Ile Asn Asp Trp Gly Arg Asn Ser Val Ile Asp Ala Gly Ala Gly Asn
 1490 1495 1500
 Asp Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly Lys
 1505 1510 1515 1520
 Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe
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 Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp
 1540 1545 1550
 Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Ile Asn Leu
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 Ser Glu Leu Trp Phe Ser Arg Glu Asn Asn Asp Leu Ile Ile Lys Ser
 1570 1575 1580
 Leu Leu Ser Glu Asp Lys Val Thr Val Gln Asn Trp Tyr Ser His Gln
 1585 1590 1595 1600
 Asp His Lys Ile Glu Asn Ile Arg Leu Ser Asn Glu Gln Thr Leu Val
 1605 1610 1615
 Ser Thr Gln Val Glu Lys Met Val Glu Ser Met Ala Gly Phe Ala Gln
 1620 1625 1630
 Lys His Gly Gly Glu Ile Ser Leu Val Ser Leu Glu Glu Val Lys Gln
 1635 1640 1645
 Tyr Ile Asn Ser Leu Thr Ala Ala Leu *
 1650 1655

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7004 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Actinobacillus pleuropneumoniae*
 - (B) STRAIN: HV114 (serotype 3 field strain)
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: pROK5
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1566..5714
 - (D) OTHER INFORMATION: /codon_start= 1566
 - /function= "RTX-toxin"
 - /product= "ApXIV_var3"
 - /gene= "apXIV_var3"
 - /number= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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ATTTCAATGT TCCAAAAGT GTCGGTACCG GTCTTAGGTA TCATTGAAAA TATGAGCGTG      180
CATATCTGCC AAAATTGCGG TCACCACGAA GATATTTTCG GCACCGGCGG TCGCGAGAAA      240
GTGGCGAAGA AATACGGTAC TAAAGTATTA GGACAAATGC CGTTGCATAT TCGCTTACGT      300
CAAGATTTGG ATGCCGGCAC ACCGACCGTC GTTGCGGCAC CGGAACACGA CACCAGCAGA      360
GCCTATATTG AATTAGCGGC AAAAGTCGCT TCGGAATTAT ACTGGCAAGG TTCGGTTATC      420
CCGTCTGAAA TTATGATTCG TGAAGTAAAA TAAGCCTACA TAACCACGGA ATACCAGATA      480
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TATTAGTACG ATTCGCTGTT GGACTGCCAT TTGATTTGGT TTGTCAGGAT ATTATGTTAT      600
TGTAATGAAA TGTTAGTGAA TTATTTTAT TAATTTGAAA GGAGACAAAA TGAAAATAAA      660
AAAACGTTAC ATTGCGCTGC TAGCTTTAGG CAGTGTTATT GGCTATGCCT GGTATCAAAA      720
TTATCAATGG GAACAGTTGA TGTTAAGTGG CTATTGTGAA AAGGACGGAA GCTATTGTGA      780
TGATAGGCAT ACGAAGCAGG AACTGATTGA TAGGGCAATT AACTATGTGC TGGAAAATCA      840
AATTCAACAG ACATATGAAG GTGATGACCT TGTGGATATA AAACAATATT CAACAATAGA      900
GGAATTTAAA AACTAAATC CGAATTGTTG TAAGGTAGAT TCTTGCCCGG ATGATGCTGT      960
TCGTGAGGAT GCTGATTTAC AGCGAGAGGG CAAAGCGTAT AAATACGTAA AAGTCAAATA     1020

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TAATTGCAGT GGAATTAAAG AAAGACACCA ACTGTATTAA AAATAGATTA GATGGAGACA	1140
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CGC TAT ATT GAA AAC TTT GGT ATT GAA CCT CTT GGT AAG CAA GAA GAT	1655
Arg Tyr Ile Glu Asn Phe Gly Ile Glu Pro Leu Gly Lys Gln Glu Asp	
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Phe Asp Phe Val Gly Gly Phe Trp Ser Asn Leu Val Asn Arg Gly Leu	
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GAA AGT ATT ATC GAC CCA TCC GGT ATC GGT GGA ACG GTA AAC CTT AAC	1751
Glu Ser Ile Ile Asp Pro Ser Gly Ile Gly Gly Thr Val Asn Leu Asn	
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Ala Glu Ala Ala Lys Lys Ser His Trp Ser Leu Val Asn Ala Ala Lys	
1740 1745 1750	
GTA TAC GGC GGT TTA GAC CAA ATT ATT AAA AAA CTA TGG GAC AGT GGC	1895
Val Tyr Gly Gly Leu Asp Gln Ile Ile Lys Lys Leu Trp Asp Ser Gly	
1755 1760 1765	
TCA ATT AAG CAT TTA TAT CAA GAT AAA GAT ACG GGC AAA TTA AAA CCG	1943
Ser Ile Lys His Leu Tyr Gln Asp Lys Asp Thr Gly Lys Leu Lys Pro	
1770 1775 1780	
ATT ATT TAC GGC ACG GCC GGC AAC GAC AGT AAG ATT GAA GGC ACT AAA	1991
Ile Ile Tyr Gly Thr Ala Gly Asn Asp Ser Lys Ile Glu Gly Thr Lys	
1785 1790 1795 1800	
ATC ACC CGT AGG ATT GCG GGT AAA GAA GTT ACG CTT GAT ATT GCC AAT	2039
Ile Thr Arg Arg Ile Ala Gly Lys Glu Val Thr Leu Asp Ile Ala Asn	
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CAG AAA ATT GAA AAA GGC GTG TTA GAG AAA TTG GGG CTG TCT GTT AGT	2087
Gln Lys Ile Glu Lys Gly Val Leu Glu Lys Leu Gly Leu Ser Val Ser	
1820 1825 1830	
GGT TCG GAT ATC ATT AAA TTG TTG TTT GGA GCA TTG ACT CCA ACT TTA	2135
Gly Ser Asp Ile Ile Lys Leu Leu Phe Gly Ala Leu Thr Pro Thr Leu	
1835 1840 1845	
AAT AGA ATG TTG CTA TCA CAA CTT ATC CAG TCT TTT TCC GAT AGC TTG	2183
Asn Arg Met Leu Leu Ser Gln Leu Ile Gln Ser Phe Ser Asp Ser Leu	
1850 1855 1860	

GCT AAA CTT GAT AAT CCC TTA GCC CCT TAC ACT AAA AAT GGC GTG GTT Ala Lys Leu Asp Asn Pro Leu Ala Pro Tyr Thr Lys Asn Gly Val Val 1865 1870 1875 1880	2231
TAT GTC ACC GGC AAA GGG AAT GAT GTG CTT AAA GGA ACT GAA CAT GAG Tyr Val Thr Gly Lys Gly Asn Asp Val Leu Lys Gly Thr Glu His Glu 1885 1890 1895	2279
GAT TTG TTT CTC GGT GGT GAG GGG AAT GAT ACT TAT TAT GCG AGA GTA Asp Leu Phe Leu Gly Gly Glu Gly Asn Asp Thr Tyr Tyr Ala Arg Val 1900 1905 1910	2327
GGC GAT ACA ATT GAA GAC GCC GAC GGC AAA GGT AAA GTC TAT TTT GTG Gly Asp Thr Ile Glu Asp Ala Asp Gly Lys Gly Lys Val Tyr Phe Val 1915 1920 1925	2375
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ACT TAC GCA GTT TTA GAA AAT TAT AAT TGG GAA GAG AAA ACG GCG ACT Thr Tyr Ala Val Leu Glu Asn Tyr Asn Trp Glu Glu Lys Thr Ala Thr 1965 1970 1975	2519
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ATT GAC GAA AAC GGA AAA GCA CTT AGC GAA AAA AGT ATT ACG GTT AAA Ile Asp Glu Asn Gly Lys Ala Leu Ser Glu Lys Ser Ile Thr Val Lys 2025 2030 2035 2040	2711
AAT TTT AAA AAT GGT GAT TTA GGA ATA AGG TTG TTG GAT CCT AAT AGC Asn Phe Lys Asn Gly Asp Leu Gly Ile Arg Leu Leu Asp Pro Asn Ser 2045 2050 2055	2759
TAT TAT TAT TTC CTT GAA GGC CAA GAT ACG GGT TTT TAT GGT CCT GCT Tyr Tyr Tyr Phe Leu Glu Gly Gln Asp Thr Gly Phe Tyr Gly Pro Ala 2060 2065 2070	2807
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GGA GCA GGA AAT AGC AAA GAT TGG GGC GGG AAC GGG CAT GGA AAT CAC Gly Ala Gly Asn Ser Lys Asp Trp Gly Gly Asn Gly His Gly Asn His 2090 2095 2100	2903
CGA AAT AAT GCC TCC GAC CTG AAT AAA CCG GAC GGA AAT AAT GGG AAT Arg Asn Asn Ala Ser Asp Leu Asn Lys Pro Asp Gly Asn Asn Gly Asn 2105 2110 2115 2120	2951
AAC CAA AAT AAC GGA AGC AAT CAA GAT AAT CAT AGC GAT GTG AAT GCG Asn Gln Asn Asn Gly Ser Asn Gln Asp Asn His Ser Asp Val Asn Ala 2125 2130 2135	2999

CCA AAT AAC CCG GGA CGT AAC TAT GAT ATT TAC GAT CCT TTA GCT TTA Pro Asn Asn Pro Gly Arg Asn Tyr Asp Ile Tyr Asp Pro Leu Ala Leu 2140 2145 2150	3047
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GGC GCG TTA TTC GAT CAT GAA GGA AAA GGT ATT CGT ACC GCA ACG GGC Gly Ala Leu Phe Asp His Glu Gly Lys Gly Ile Arg Thr Ala Thr Gly 2170 2175 2180	3143
TGG CTC GCT GCG GAT GAC GGT TTT TTA GTG TTA GAT CGT AAC CAA GAC Trp Leu Ala Ala Asp Asp Gly Phe Leu Val Leu Asp Arg Asn Gln Asp 2185 2190 2195 2200	3191
GGC ATT ATT AAT GAT ATA AGC GAG TTA TTT AGT AAT AAA AAT CAA CTT Gly Ile Ile Asn Asp Ile Ser Glu Leu Phe Ser Asn Lys Asn Gln Leu 2205 2210 2215	3239
TCC GAC GGG AGT ATT TCT GCA CAC GGT TTT GCG ACA TTA GCC GAT TTG Ser Asp Gly Ser Ile Ser Ala His Gly Phe Ala Thr Leu Ala Asp Leu 2220 2225 2230	3287
GAT ACA AAC CAA GAT CAG CGT ATC GAC CAA AAT GAT AAG CTG TTT TCT Asp Thr Asn Gln Asp Gln Arg Ile Asp Gln Asn Asp Lys Leu Phe Ser 2235 2240 2245	3335
AAA CTC CAA ATT TGG CGG GAT TTA AAT CAA AAC GGT TTT AGT GAA GCG Lys Leu Gln Ile Trp Arg Asp Leu Asn Gln Asn Gly Phe Ser Glu Ala 2250 2255 2260	3383
AAT GAG CTG TTT AGC TTA GAA AGT TTG AAT ATT AAA TCT TTA CAT ACC Asn Glu Leu Phe Ser Leu Glu Ser Leu Asn Ile Lys Ser Leu His Thr 2265 2270 2275 2280	3431
GCC TAT GAA GAG CGT AAT GAT TTT CTA GCG GGC AAT AAT ATC CTT GCT Ala Tyr Glu Glu Arg Asn Asp Phe Leu Ala Gly Asn Asn Ile Leu Ala 2285 2290 2295	3479
CAG CTT GGG AAG TAT GAA AAA ACG GAC GGT ACT TTT GGA CAA ATG GGC Gln Leu Gly Lys Tyr Glu Lys Thr Asp Gly Thr Phe Gly Gln Met Gly 2300 2305 2310	3527
GAT TTA AAT TTC AGT TTT AAC CCG TTT TAT AGC CGA TTT ACC GAA GCG Asp Leu Asn Phe Ser Phe Asn Pro Phe Tyr Ser Arg Phe Thr Glu Ala 2315 2320 2325	3575
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GGT CGG GTT CGG GAT TTG CGT GAA GCC GCC GCA CTT TCT GAG GAG TTG Gly Arg Val Arg Asp Leu Arg Glu Ala Ala Ala Leu Ser Glu Glu Leu 2345 2350 2355 2360	3671
GCT GCT TTA TTA CAA CAG TAC ACT AAG GGC TCC GAT TTT CAG GCA CAA Ala Ala Leu Leu Gln Gln Tyr Thr Lys Gly Ser Asp Phe Gln Ala Gln 2365 2370 2375	3719
CGA GAA TTA TTG CCT GCC ATT TTA GAT AAA TGG GCG GCA ACG GAT TTA Arg Glu Leu Leu Pro Ala Ile Leu Asp Lys Trp Ala Ala Thr Asp Leu 2380 2385 2390	3767
CAG TAT CAA CAT TAT GAT AAA ACA TTA CTT AAA ACG GTA GAA AGT ACC Gln Tyr Gln His Tyr Asp Lys Thr Leu Leu Lys Thr Val Glu Ser Thr 2395 2400 2405	3815

GAT AGT AGT GCT TCT GTC GTT AGA GTC ACG CCT TCT CAA TTA AGT AGT Asp Ser Ser Ala Ser Val Val Arg Val Thr Pro Ser Gln Leu Ser Ser 2410 2415 2420	3863
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AGT AAG GCA AAA ATT GCG ACT TTA AAT TCG CTC TAC GGG TTA AAT ATT Ser Lys Ala Lys Ile Ala Thr Leu Asn Ser Leu Tyr Gly Leu Asn Ile 2445 2450 2455	3959
GAT CAA CTT TAT TAT ACG ACG GAT AAA GAC ATT CGT TAT ATT ACT GAC Asp Gln Leu Tyr Tyr Thr Thr Asp Lys Asp Ile Arg Tyr Ile Thr Asp 2460 2465 2470	4007
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TTT CGA CGA GTA GAT AAT GAC TTA ATG TTA TTC GGT TAT CAT GAT ACG Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His Asp Thr 2685 2690 2695	4679
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GAC AAA TTG GAG TTT GCT GAC CGC AGT ATA ACT CGC GAT GAA CTG ATT Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu Ile 2715 2720 2725	4775
AAA GCA GGG CTT CAT CTA TAC GGC ACC GAT GGC AAT GAT GAT ATA AAG Lys Ala Gly Leu His Leu Tyr Gly Thr Asp Gly Asn Asp Asp Ile Lys 2730 2735 2740	4823
GAT CAT GCG GAT TGG GAC AGC ATT GTG GAA GGC GGC AAA GGC AAC GAT Asp His Ala Asp Trp Asp Ser Ile Val Glu Gly Gly Lys Gly Asn Asp 2745 2750 2755 2760	4871
ATT CTA AGA GGT GGC TAC GGT GCG GAC ACC TAT ATC TTT AGC AAA GGA Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly 2765 2770 2775	4919
CAC GGA CAG GAT ATC GTT TAT GAA GAT ACC AAT AAT GAT AAC CGA GCA His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg Ala 2780 2785 2790	4967
AGA GAT ATC GAC ACC TTA ACA TTT ACT GAT GTG AAT TAT GCG GAA GTG Arg Asp Ile Asp Thr Leu Thr Phe Thr Asp Val Asn Tyr Ala Glu Val 2795 2800 2805	5015
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ACG GAT TCG GTC ACG ATA AAA TCC TTC TAC AAC CAT GTA GAT TAT CAA Thr Asp Ser Val Thr Ile Lys Ser Phe Tyr Asn His Val Asp Tyr Gln 2825 2830 2835 2840	5111
TGT GAC AAA TTG GAC TTT GCT GAC CGC AGT ATA ACT CGT GAT GAA CTA Cys Asp Lys Leu Asp Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu 2845 2850 2855	5159
GGT AAA CAA GGT ATG GCA TTA TTT GGC ACT GAC GGC GAT GAT AAT ATC Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp Asn Ile 2860 2865 2870	5207
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ACG GTT AAT GGC GGT AAT GGC GAT GAC ACC CTC ATC GGC GGC AAA GGT Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly Lys Gly 2890 2895 2900	5303
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CGC GCA AGA GAT ATC GAC ACC TTA AAA TTT ACT GAT ATT AAT TTA TCC Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Ile Asn Leu Ser 2940 2945 2950	5447

GAA CTT TGG TTT AGC CGA GAA AAT AAC GAT TTG ATT ATT AAA TCA TTA Glu Leu Trp Phe Ser Arg Glu Asn Asn Asp Leu Ile Ile Lys Ser Leu 2955 2960 2965	5495
TTA AGT GAG GAT AAA GTC ACG GTT CAA AAT TGG TAT TCA CAC CAA GAT Leu Ser Glu Asp Lys Val Thr Val Gln Asn Trp Tyr Ser His Gln Asp 2970 2975 2980	5543
CAT AAA ATA GAA AAT ATT CGT TTA TCG AAT GAG CAA ATG TTG GTG AGC His Lys Ile Glu Asn Ile Arg Leu Ser Asn Glu Gln Met Leu Val Ser 2985 2990 2995 3000	5591
ACT CAG GTG GAG AAG ATG GTT GAG TCG ATG GCC GGC TTT GCT CAG AAG Thr Gln Val Glu Lys Met Val Glu Ser Met Ala Gly Phe Ala Gln Lys 3005 3010 3015	5639
CAC GGA GGA GAG ATA TCT CTT CTG TCG CCT GAA GAG GTA AAA CAA TAT His Gly Gly Glu Ile Ser Leu Leu Ser Pro Glu Glu Val Lys Gln Tyr 3020 3025 3030	5687
ATC AAT AGC TTA ACA GCT GCT TTA TAA CATACGAAAG AAATCGGCAC Ile Asn Ser Leu Thr Ala Ala Leu * 3035 3040	5734
AGTTTTTGTG AACTGTGCCG ATTTGATTTT AGTGTAAGAA TATAGCCTGA TTTTAAGAAA	5794
TTTACTCTTG GCTAATAACT ATTTCCCATTT TTATAAGTTA TTGACGGATG GTTTTATCAA	5854
ATATGAGATC AAATCTTATT TTAAATTCGC TTTCCATTAA GCGATATTGA TCTTTTAAGT	5914
TTGGGGCCGC ATGAGTTTGG AACCGATACC ACTCATTTGTG GGAATCAATA CACAATACGC	5974
TGTAATCGGA CTCTTGCACT TCATAATAAT GCTTTCTCTC CGTTAATTCT TCTTGCGTAT	6034
ATGGCGAGAG ATTAAAGCTG AATGGCTGGT TCGCACTAAC AAACAGGTTT TCCGATTTCA	6094
GATATTCACA ACCGTAATGG CTACCGGTTT CCTGCGGTTT TACATAATTG GTATGATTTT	6154
GTTTAGCTGT TATACGGTAG ATGCCTAATT GTGGTAAATT GCGTGTGTCA ATATAGCTTT	6214
CTTGTTCTCC GTAACCGAAA TACTCAATGG CGTTTTCTGT TTTAGCTAAG AAGAAACGTA	6274
AGCCGAAGCG GGGTAAATAC GGTAATTCGA TCGGGCGAAT AGCGTTAATT TCAACCGAAA	6334
GTTGTCCGTC ATTGAAGATA CGATAACGAA TATCCAGTGT TAAATGCGA CCGCGAGAAA	6394
TTGACACAAT TGCAGATTTT ACTGAAAATT CGACCGCTTG TTCGCTTTGC TGCCACTGAA	6454
TTTCATACGC TCTGGTATAG GCTTTATCGT AGCCGGCATT TTGGCACGCC TCACGAATGA	6514
GGCGATCATT GTCGGTTGGC GCACGCCAAA TATTAAAATC TAACGATTGT TGGATAATCG	6574
CTTTACCGGC TTTTCAATA CGGGTGAAAA TCCCTTTCTG TTTATCTAAT TGATAACTAA	6634
ATTGACCGTT GTGTACGTTA ATGTGGAAGC GATCTTCTTG TACTTCAAAT GCACTGTTCT	6694
CAATTGTGAA TTGTGGTAAT ACTAATTTAT TTTCGCTAAA TAAATTGAGC TGCTCGAAGC	6754
CAAGTGAATG TGCTTCGTCT AATAATTCGA CCGCGGTATT TAAGCGATAA TTTAAATTCA	6814
GTAGCCATAA ATGCCCGTTA TTTTTTGGTA ACTCAATCGG TAATACTACG CTGCCGTGCG	6874
GTGGCAAGA AACCGATAAA TTCCCACCGC TTGTCACCAC GCCGTTTTCG ACAAATTCGT	6934
AATCAATCGT TAAATAATCG GCAAGATCAG TGAAATCCAA GTAGTTGTGG ATCACAATTT	6994
GGTTATCGAT	7004

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1383 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Met Ser Asp Asn Ala Phe Phe Val Ile Glu Glu Ser Gly Lys Arg Tyr
 1             5             10             15

Ile Glu Asn Phe Gly Ile Glu Pro Leu Gly Lys Gln Glu Asp Phe Asp
      20             25             30

Phe Val Gly Gly Phe Trp Ser Asn Leu Val Asn Arg Gly Leu Glu Ser
      35             40             45

Ile Ile Asp Pro Ser Gly Ile Gly Gly Thr Val Asn Leu Asn Phe Thr
      50             55             60

Gly Glu Val Glu Thr Tyr Thr Leu Asp Glu Thr Arg Phe Lys Ala Glu
      65             70             75             80

Ala Ala Lys Lys Ser His Trp Ser Leu Val Asn Ala Ala Lys Val Tyr
      85             90             95

Gly Gly Leu Asp Gln Ile Ile Lys Lys Leu Trp Asp Ser Gly Ser Ile
      100            105            110

Lys His Leu Tyr Gln Asp Lys Asp Thr Gly Lys Leu Lys Pro Ile Ile
      115            120            125

Tyr Gly Thr Ala Gly Asn Asp Ser Lys Ile Glu Gly Thr Lys Ile Thr
      130            135            140

Arg Arg Ile Ala Gly Lys Glu Val Thr Leu Asp Ile Ala Asn Gln Lys
      145            150            155            160

Ile Glu Lys Gly Val Leu Glu Lys Leu Gly Leu Ser Val Ser Gly Ser
      165            170            175

Asp Ile Ile Lys Leu Leu Phe Gly Ala Leu Thr Pro Thr Leu Asn Arg
      180            185            190

Met Leu Leu Ser Gln Leu Ile Gln Ser Phe Ser Asp Ser Leu Ala Lys
      195            200            205

Leu Asp Asn Pro Leu Ala Pro Tyr Thr Lys Asn Gly Val Val Tyr Val
      210            215            220

Thr Gly Lys Gly Asn Asp Val Leu Lys Gly Thr Glu His Glu Asp Leu
      225            230            235            240

Phe Leu Gly Gly Glu Gly Asn Asp Thr Tyr Tyr Ala Arg Val Gly Asp
      245            250            255

Thr Ile Glu Asp Ala Asp Gly Lys Gly Lys Val Tyr Phe Val Arg Glu
      260            265            270

Lys Gly Val Pro Lys Ala Asp Pro Lys Arg Val Glu Phe Ser Glu Tyr
      275            280            285

Ile Thr Lys Glu Glu Ile Lys Glu Val Glu Lys Gly Leu Leu Thr Tyr
      290            295            300

Ala Val Leu Glu Asn Tyr Asn Trp Glu Glu Lys Thr Ala Thr Phe Ala

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His Ala Thr Met Leu Asn Glu Leu Phe Thr Asp Tyr Thr Asn Tyr Arg
 325 330 335
 Tyr Glu Val Lys Gly Leu Lys Leu Pro Ala Val Lys Lys Leu Lys Ser
 340 345 350
 Pro Leu Val Glu Phe Thr Ala Asp Leu Leu Thr Val Thr Pro Ile Asp
 355 360 365
 Glu Asn Gly Lys Ala Leu Ser Glu Lys Ser Ile Thr Val Lys Asn Phe
 370 375 380
 Lys Asn Gly Asp Leu Gly Ile Arg Leu Leu Asp Pro Asn Ser Tyr Tyr
 385 390 395 400
 Tyr Phe Leu Glu Gly Gln Asp Thr Gly Phe Tyr Gly Pro Ala Phe Tyr
 405 410 415
 Ile Glu Arg Lys Asn Gly Gly Gly Ser Lys Asn Asn Ser Ser Gly Ala
 420 425 430
 Gly Asn Ser Lys Asp Trp Gly Gly Asn Gly His Gly Asn His Arg Asn
 435 440 445
 Asn Ala Ser Asp Leu Asn Lys Pro Asp Gly Asn Asn Gly Asn Asn Gln
 450 455 460
 Asn Asn Gly Ser Asn Gln Asp Asn His Ser Asp Val Asn Ala Pro Asn
 465 470 475 480
 Asn Pro Gly Arg Asn Tyr Asp Ile Tyr Asp Pro Leu Ala Leu Asp Leu
 485 490 495
 Asp Gly Asp Gly Leu Glu Thr Val Ser Met Asn Gly Arg Gln Gly Ala
 500 505 510
 Leu Phe Asp His Glu Gly Lys Gly Ile Arg Thr Ala Thr Gly Trp Leu
 515 520 525
 Ala Ala Asp Asp Gly Phe Leu Val Leu Asp Arg Asn Gln Asp Gly Ile
 530 535 540
 Ile Asn Asp Ile Ser Glu Leu Phe Ser Asn Lys Asn Gln Leu Ser Asp
 545 550 555 560
 Gly Ser Ile Ser Ala His Gly Phe Ala Thr Leu Ala Asp Leu Asp Thr
 565 570 575
 Asn Gln Asp Gln Arg Ile Asp Gln Asn Asp Lys Leu Phe Ser Lys Leu
 580 585 590
 Gln Ile Trp Arg Asp Leu Asn Gln Asn Gly Phe Ser Glu Ala Asn Glu
 595 600 605
 Leu Phe Ser Leu Glu Ser Leu Asn Ile Lys Ser Leu His Thr Ala Tyr
 610 615 620
 Glu Glu Arg Asn Asp Phe Leu Ala Gly Asn Asn Ile Leu Ala Gln Leu
 625 630 635 640
 Gly Lys Tyr Glu Lys Thr Asp Gly Thr Phe Gly Gln Met Gly Asp Leu
 645 650 655
 Asn Phe Ser Phe Asn Pro Phe Tyr Ser Arg Phe Thr Glu Ala Leu Asn
 660 665 670
 Leu Thr Glu Gln Gln Arg Arg Thr Ile Asn Leu Thr Gly Thr Gly Arg
 675 680 685

Val Arg Asp Leu Arg Glu Ala Ala Ala Leu Ser Glu Glu Leu Ala Ala
 690 695 700
 Leu Leu Gln Gln Tyr Thr Lys Gly Ser Asp Phe Gln Ala Gln Arg Glu
 705 710 715 720
 Leu Leu Pro Ala Ile Leu Asp Lys Trp Ala Ala Thr Asp Leu Gln Tyr
 725 730 735
 Gln His Tyr Asp Lys Thr Leu Leu Lys Thr Val Glu Ser Thr Asp Ser
 740 745 750
 Ser Ala Ser Val Val Arg Val Thr Pro Ser Gln Leu Ser Ser Ile Arg
 755 760 765
 Asn Val Lys His Asp Pro Thr Val Met Gln Asn Cys Glu Gln Ser Lys
 770 775 780
 Ala Lys Ile Ala Thr Leu Asn Ser Leu Tyr Gly Leu Asn Ile Asp Gln
 785 790 795 800
 Leu Tyr Tyr Thr Thr Asp Lys Asp Ile Arg Tyr Ile Thr Asp Lys Val
 805 810 815
 Asn Asn Met Tyr Gln Thr Thr Gly Glu Leu Gly Tyr Arg Ser Leu Leu
 820 825 830
 Leu Gln Thr Arg Val Lys Lys Tyr Val Tyr Ser Val Asn Ala Lys Gln
 835 840 845
 Phe Glu Gly Lys Trp Val Ala Asp Tyr Ser Arg Thr Glu Ala Leu Phe
 850 855 860
 Asn Ser Thr Tyr Lys Gln Ser Pro Glu Asn Val Leu Tyr Asp Leu Arg
 865 870 875 880
 Glu Tyr Leu Ser Phe Tyr Asn Asp Pro Thr Glu Trp Lys Glu Gly Leu
 885 890 895
 Leu Leu Leu Ser Arg Tyr Ile Asp Tyr Ala Lys Ala Gln Gly Phe Tyr
 900 905 910
 Glu Asn Trp Ala Ala Thr Ser Asn Leu Thr Ile Ala Arg Leu Arg Glu
 915 920 925
 Ala Gly Val Ile Cys Ala Glu Ser Thr Asp Leu Lys Gly Asp Glu Lys
 930 935 940
 Asn Asn Ile Val Leu Gly Ser Gln Lys Asp Asn Asn Leu Ser Gly Ser
 945 950 955 960
 Ala Gly Asp Asp Leu Leu Ile Gly Gly Glu Gly Asn Asp Thr Leu Lys
 965 970 975
 Gly Ser Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly His Gly Gln
 980 985 990
 Asp Val Ile Tyr Glu Tyr Ser Asp Ser Ala Asn Ser Lys Lys Asp Ile
 995 1000 1005
 Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala Glu Val Lys Phe Arg
 1010 1015 1020
 Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His Asp Thr Asp Ser
 1025 1030 1035 1040
 Val Thr Val Lys Ser Phe Tyr Ser His Val Asp Tyr Gln Phe Asp Lys
 1045 1050 1055

Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu Ile Lys Ala
 1060 1065 1070
 Gly Leu His Leu Tyr Gly Thr Asp Gly Asn Asp Asp Ile Lys Asp His
 1075 1080 1085
 Ala Asp Trp Asp Ser Ile Val Glu Gly Gly Lys Gly Asn Asp Ile Leu
 1090 1095 1100
 Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly His Gly
 1105 1110 1115 1120
 Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg Ala Arg Asp
 1125 1130 1135
 Ile Asp Thr Leu Thr Phe Thr Asp Val Asn Tyr Ala Glu Val Lys Phe
 1140 1145 1150
 Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His Asp Thr Asp
 1155 1160 1165
 Ser Val Thr Ile Lys Ser Phe Tyr Asn His Val Asp Tyr Gln Cys Asp
 1170 1175 1180
 Lys Leu Asp Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu Leu Gly Lys
 1185 1190 1195 1200
 Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp Asn Ile Asn Asp
 1205 1210 1215
 Trp Gly Arg Asn Ser Val Ile Asp Ala Gly Ala Gly Asn Asp Thr Val
 1220 1225 1230
 Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly Lys Gly Asn Asp
 1235 1240 1245
 Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys Gly
 1250 1255 1260
 His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg Ala
 1265 1270 1275 1280
 Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Ile Asn Leu Ser Glu Leu
 1285 1290 1295
 Trp Phe Ser Arg Glu Asn Asn Asp Leu Ile Ile Lys Ser Leu Leu Ser
 1300 1305 1310
 Glu Asp Lys Val Thr Val Gln Asn Trp Tyr Ser His Gln Asp His Lys
 1315 1320 1325
 Ile Glu Asn Ile Arg Leu Ser Asn Glu Gln Met Leu Val Ser Thr Gln
 1330 1335 1340
 Val Glu Lys Met Val Glu Ser Met Ala Gly Phe Ala Gln Lys His Gly
 1345 1350 1355 1360
 Gly Glu Ile Ser Leu Leu Ser Pro Glu Glu Val Lys Gln Tyr Ile Asn
 1365 1370 1375
 Ser Leu Thr Ala Ala Leu *
 1380

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6736 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Actinobacillus pleuropneumoniae
 - (B) STRAIN: 4074 (serotype 1 reference strain)
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: pROK7
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:1132..6549
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION:/codon_start= 1132
/function= "RTX toxin"
/product= "ApxIV"
/evidence= EXPERIMENTAL
/gene= "ApxIV_v1"
- (ix). FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:639..1178
 - (D) OTHER INFORMATION:/codon_start= 639
/function= "unknown"
/product= "ORF1"
/gene= "orf1"
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:1..453
 - (D) OTHER INFORMATION:/partial
/product= "Met-G"
/gene= "mrp"
/standard_name= "mrp"
/label= mrp
- (ix) FEATURE:
 - (A) NAME/KEY: -10_signal
 - (B) LOCATION:617..623
 - (D) OTHER INFORMATION:/standard_name= "-10"
/label= -10_s
- (ix) FEATURE:
 - (A) NAME/KEY: -35_signal
 - (B) LOCATION:594..599
 - (D) OTHER INFORMATION:/standard_name= "-35_s"
/label= -35_s
- (ix) FEATURE:
 - (A) NAME/KEY: promoter
 - (B) LOCATION:454..1131
 - (D) OTHER INFORMATION:/function= "Promoter"
/standard_name= "promoter ApxIV"
/label= pPromoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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Ile	Asp	Met	Pro	Pro	Gly	Thr	Gly	Asp	Ile	Gln	Leu	Thr	Leu	Ser	Gln		
1				5					10					15			
CAA	ATT	CCG	GTT	ACC	GGT	GCG	GTG	GTG	GTA	ACC	ACT	CCG	CAA	GAT	ATT		96
Gln	Ile	Pro	Val	Thr	Gly	Ala	Val	Val	Val	Thr	Thr	Pro	Gln	Asp	Ile		
			20					25					30				
GCG	TTA	TTA	GAT	GCG	GTG	AAA	GGT	ATT	TCA	ATG	TTC	CAA	AAA	GTG	TCG		144
Ala	Leu	Leu	Asp	Ala	Val	Lys	Gly	Ile	Ser	Met	Phe	Gln	Lys	Val	Ser		
			35				40					45					
GTA	CCG	GTC	TTA	GGT	ATC	ATT	GAA	AAT	ATG	AGC	GTA	CAT	ATC	TAC	CAA		100

AAT TGC GGT CAC CAC GAA GAT ATT TTC GGC ACC GGC GGT GCG GAG AAA Asn Cys Gly His His Glu Asp Ile Phe Gly Thr Gly Gly Ala Glu Lys 65 70 75 80	240
GTG GCG AAG AAA TAC GGT ACT AAA GTA TTA GGA CAA ATG CCG TTG CAT Val Ala Lys Lys Tyr Gly Thr Lys Val Leu Gly Gln Met Pro Leu His 85 90 95	288
ATT CGC TTA CGT CAA GAT TTG GAT GCC GGC ACA CCG ACC GTC GTT GCG Ile Arg Leu Arg Gln Asp Leu Asp Ala Gly Thr Pro Thr Val Val Ala 100 105 110	336
GCA CCG GAA CAC GAA ACC AGC CGA GCC TAT ATT GAA TTA GCG GCA AAA Ala Pro Glu His Glu Thr Ser Arg Ala Tyr Ile Glu Leu Ala Ala Lys 115 120 125	384
GTC GCT TCG GAA TTA TAC TGG CAA GGT TCG GTT ATC CCG TCT GAA ATT Val Ala Ser Glu Leu Tyr Trp Gln Gly Ser Val Ile Pro Ser Glu Ile 130 135 140	432
ATG ATT CGT GAA GTA AAA TAA GTTTTAATAA CCACGAAAAC ACAAAGAACA Met Ile Arg Glu Val Lys * 145 150	483
CAAGCGGTAG AATTTGCAGA AAAATTTGCA AATCCTACCG CTTTTTTATT AGTACGATTC	543
GCTGTTGGAC TGCTATTTGA TTTGGTTTGT CAGGATATTA TGTTATTGTA ATGAAATGTT	603
AGTGAATTAT TTTTATTAAT TTGAAAGGAA ACAAATGAA AATAAAAAAA CGTTACATTG	663
CGCTGTTGGT CTTAGGTGTC GTTATCAGCT ATGCCTGGTA TCAAAATTAT CAATGGGAAC	723
AGCTGATGTT AAGCGGTTAT TGTGAAAAGG ACGGAAGTTA TTTTGATGAT AGGCATACGA	783
AGCAAGAACT GATTGATAGG GCAATTAACAT ATATGCTGGA GCATCAATCT AAAAAACAT	843
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AAAAACTCAA TCCAAATTGT TGTGAAATTA CCTCATGGCC AGCAGATGCA GTTCCACAAG	963
ATTGGGATGT TCGTGTGGAA GGTAAGGCAT ATAGGTATGT AATCGTAAAA TATTTAAGAA	1023
CCTTAGCAAA TAGAGAACCT GAACGATGGG AAACAGTAT TGTTTTGTAT AATTGCGGCA	1083
ATCCTAAAAG AGCAAGCTAC TTATATTATT TAAAGAGAGA AATTTATT ATG ACA AAA Met Thr Lys 1	1140
TTA ACT ATG CAA GAT GTG ACC AAT TTA TAT TTA TAT AAA ACG AAA ACT Leu Thr Met Gln Asp Val Thr Asn Leu Tyr Leu Tyr Lys Thr Lys Thr 5 10 15	1188
CTA CCT AAA GAT AGA TTG GAT GAT TCA CTT ATT TCT GAA ATA GGA AAA Leu Pro Lys Asp Arg Leu Asp Asp Ser Leu Ile Ser Glu Ile Gly Lys 20 25 30 35	1236
GGA GAT GAT GAT ATT GAT AGA AAA GAA TTT ATG GTG GGG CCG GGA CGT Gly Asp Asp Asp Ile Asp Arg Lys Glu Phe Met Val Gly Pro Gly Arg 40 45 50	1284
TTT GTG ACC GCT GAT AAC TTT AGC GTT GTA AGA GAT TTT TTT AAT GCT Phe Val Thr Ala Asp Asn Phe Ser Val Val Arg Asp Phe Phe Asn Ala 55 60 65	1332
GGG AAA TCA CGC ATT ATT GCG CCG CAA GTC CCG CCT ATT CGT TCA CAG Gly Lys Ser Arg Ile Ile Ala Pro Gln Val Pro Pro Ile Arg Ser Gln 70 75 80	1380
CAG GAA AAA ATC TTG GTC GGT TTA AAA CCG GGC AAA TAT TCC AAA GCG Gln Glu Lys Ile Leu Val Gly Leu Lys Pro Gly Lys Tyr Ser Lys Ala 85 90 95	1428
CAG ATA TTG GAA ATG CTG GGT TAT ACG AAA GGC GGA GAA GTG GTA AAT Gln Ile Leu Glu Met Leu Gly Tyr Thr Lys Gly Gly Glu Val Val Asn 100 105 110 115	1476
GGC ATG TTT GCC GGT GAA GTC CAG ACA TTA GGC TTT TAT GAC GAT GGC Gly Met Phe Ala Gly Glu Val Gln Thr Leu Gly Phe Tyr Asp Asp Gly 120 125 130	1524
AAA GGG GAT TTA CTC GAA CGC GCC TAT ATC TGG AAT ACC ACA GGA TTT Lys Gly Asp Leu Leu Glu Arg Ala Tyr Ile Trp Asn Thr Thr Gly Phe 135 140 145	1572

Lys	Met	Ser	Asp	Asn	Ala	Phe	Phe	Val	Ile	Glu	Glu	Ser	Gly	Lys	Arg	
		150					155					160				
TAT	ATT	GAA	AAC	TTT	GGT	ATT	GAA	CCT	CTT	GGT	AAG	CAA	GAA	GAT	TTT	1668
Tyr	Ile	Glu	Asn	Phe	Gly	Ile	Glu	Pro	Leu	Gly	Lys	Gln	Glu	Asp	Phe	
	165					170					175					
GAT	TTT	GTC	GGC	GGC	TTT	TGG	TCT	AAC	TTA	GTG	AAT	CGT	GGT	TTG	GAA	1716
Asp	Phe	Val	Gly	Gly	Phe	Trp	Ser	Asn	Leu	Val	Asn	Arg	Gly	Leu	Glu	
	180				185					190					195	
AGT	ATT	ATC	GAC	CCA	TCC	GGT	ATC	GGT	GGA	ACG	GTA	AAC	CTT	AAC	TTT	1764
Ser	Ile	Ile	Asp	Pro	Ser	Gly	Ile	Gly	Gly	Thr	Val	Asn	Leu	Asn	Phe	
				200					205					210		
ACC	GGC	GAG	GTG	GAA	ACC	TAC	ACG	TTA	GAC	GAA	ACA	AGG	TTT	AAA	GCG	1812
Thr	Gly	Glu	Val	Glu	Thr	Tyr	Thr	Leu	Asp	Glu	Thr	Arg	Phe	Lys	Ala	
			215					220					225			
GAA	GCG	GCG	AAG	AAA	AGC	CAT	TGG	AGT	TTA	GTG	AAT	GCG	GCG	AAA	GTA	1860
Glu	Ala	Ala	Lys	Lys	Ser	His	Trp	Ser	Leu	Val	Asn	Ala	Ala	Lys	Val	
	230						235					240				
TAC	GGC	GGT	TTA	GAC	CAA	ATT	ATT	AAA	AAA	CTA	TGG	GAC	AGT	GGC	TCA	1908
Tyr	Gly	Gly	Leu	Asp	Gln	Ile	Ile	Lys	Lys	Leu	Trp	Asp	Ser	Gly	Ser	
	245					250					255					
ATT	AAG	CAT	TTA	TAT	CAA	GAT	AAA	GAT	ACG	GGC	AAA	TTA	AAA	CCG	ATT	1956
Ile	Lys	His	Leu	Tyr	Gln	Asp	Lys	Asp	Thr	Gly	Lys	Leu	Lys	Pro	Ile	
	260				265					270					275	
ATT	TAC	GGC	ACG	GCC	GGC	AAC	GAC	AGT	AAG	ATT	GAA	GGC	ACT	AAA	ATC	2004
Ile	Tyr	Gly	Thr	Ala	Gly	Asn	Asp	Ser	Lys	Ile	Glu	Gly	Thr	Lys	Ile	
				280					285					290		
ACC	CGT	AGG	ATT	GCG	GGT	AAA	GAA	GTT	ACG	CTT	GAT	ATT	GCC	AAT	CAG	2052
Thr	Arg	Arg	Ile	Ala	Gly	Lys	Glu	Val	Thr	Leu	Asp	Ile	Ala	Asn	Gln	
			295					300					305			
AAA	ATT	GAA	AAA	GGC	GTG	TTA	GAG	AAA	TTG	GGG	CTG	TCT	GTT	AGT	GGT	2100
Lys	Ile	Glu	Lys	Gly	Val	Leu	Glu	Lys	Leu	Gly	Leu	Ser	Val	Ser	Gly	
		310					315					320				
TCG	GAT	ATC	ATT	AAA	TTG	TTG	TTT	GGA	GCA	TTG	ACT	CCA	ACT	TTA	AAT	2148
Ser	Asp	Ile	Ile	Lys	Leu	Leu	Phe	Gly	Ala	Leu	Thr	Pro	Thr	Leu	Asn	
	325					330					335					
AGA	ATG	TTG	CTA	TCA	CAA	CTT	ATC	CAG	TCT	TTT	TCC	GAT	AGC	TTG	GCT	2196
Arg	Met	Leu	Leu	Ser	Gln	Leu	Ile	Gln	Ser	Phe	Ser	Asp	Ser	Leu	Ala	
	340				345					350					355	
AAA	CTT	GAT	AAT	CCC	TTA	GCC	CCT	TAC	ACT	AAA	AAT	GGC	GTG	GTT	TAT	2244
Lys	Leu	Asp	Asn	Pro	Leu	Ala	Pro	Tyr	Thr	Lys	Asn	Gly	Val	Val	Tyr	
				360					365					370		
GTC	ACC	GGC	AAA	GGG	AAT	GAT	GTG	CTT	AAA	GGA	ACT	GAA	CAT	GAG	GAT	2292
Val	Thr	Gly	Lys	Gly	Asn	Asp	Val	Leu	Lys	Gly	Thr	Glu	His	Glu	Asp	
			375					380					385			
TTG	TTT	CTC	GGT	GGT	GAG	GGG	AAT	GAT	ACT	TAT	TAT	GCG	AGA	GTA	GGC	2340
Leu	Phe	Leu	Gly	Gly	Glu	Gly	Asn	Asp	Thr	Tyr	Tyr	Ala	Arg	Val	Gly	
		390					395					400				
GAT	ACA	ATT	GAA	GAC	GCC	GAC	GGC	AAA	GGT	AAA	GTC	TAT	TTT	GTG	AGA	2388
Asp	Thr	Ile	Glu	Asp	Ala	Asp	Gly	Lys	Gly	Lys	Val	Tyr	Phe	Val	Arg	
	405					410					415					
GAA	AAA	GGG	GTA	CCT	AAG	GCG	GAT	CCT	AAG	CGG	GTA	GAG	TTT	AGC	GAG	2436
Glu	Lys	Gly	Val	Pro	Lys	Ala	Asp	Pro	Lys	Arg	Val	Glu	Phe	Ser	Glu	
	420				425					430					435	
TAC	ATA	ACG	AAA	GAA	GAA	ATA	AAA	GAG	GTT	GAA	AAG	GGG	TTA	TTA	ACT	2484
Tyr	Ile	Thr	Lys	Glu	Glu	Ile	Lys	Glu	Val	Glu	Lys	Gly	Leu	Leu	Thr	
				440					445					450		
TAC	GCA	GTT	TTA	GAA	AAT	TAT	AAT	TGG	GAA	GAG	AAA	ACG	GCG	ACT	TTC	2532
Tyr	Ala	Val	Leu	Glu	Asn	Tyr	Asn	Trp	Glu	Glu	Lys	Thr	Ala	Thr	Phe	
			455					460					465			
GCT	CAT	GCG	ACT	ATG	CTT	AAT	GAG	CTT	TTT	ACT	GAT	TAT	ACT	AAT	TAT	2580
Ala	His	Ala	Thr	Met	Leu	Asn	Glu	Leu	Phe	Thr	Asp	Tyr	Thr	Asn	Tyr	
		470					475					480				

485	490	495	
AGT CCG TTG GTG GAG TTT ACA GCT GAT TTA TTA ACT GTT ACG CCT ATT Ser Pro Leu Val Glu Phe Thr Ala Asp Leu Leu Thr Val Thr Pro Ile 500 505 510 515			2676
GAC GAA AAC GGA AAA GCA CTT AGC GAA AAA AGT ATT ACG GTT AAA AAT Asp Glu Asn Gly Lys 520 Ala Leu Ser Glu Lys 525 Ser Ile Thr Val Lys Asn 530			2724
TTT AAA AAT GGT GAT TTA GGA ATA AGG TTG TTG GAT CCT AAT AGC TAT Phe Lys Asn Gly Asp Leu Gly Ile Arg Leu Leu Asp Pro Asn Ser Tyr 535 540 545			2772
TAT TAT TTC CTT GAA GGC CAA GAT ACG GGT TTT TAT GGT CCT GCT TTT Tyr Tyr Phe Leu Glu Gly Gln Asp Thr Gly Phe Tyr Gly Pro Ala Phe 550 555 560			2820
TAT ATT GAA CGA AAA AAC GGT GGC GCT AAA AAT AAC TCG TCG GGA Tyr Ile Glu Arg Lys Asn Gly Gly Ala Lys Asn Asn Ser Ser Gly 565 570 575			2868
GCA GGA AAT AGC AAA GAT TGG GGC GGG AAC GGG CAT GGA AAT CAC CGA Ala Gly Asn Ser Lys Asp Trp Gly Gly Asn Gly His Gly Asn His Arg 580 585 590 595			2916
AAT AAT GCC TCC GAC CTG AAT AAA CCG GAC GGA AAT AAT GGG AAT AAC Asn Asn Ala Ser Asp Leu Asn Lys Pro Asp Gly Asn Asn Gly Asn Asn 600 605 610			2964
CAA AAT AAC GGA AGC AAT CAA GAT AAT CAT AGC GAT GTG AAT GCG CCA Gln Asn Asn Gly Ser Asn Gln Asp Asn His Ser Asp Val Asn Ala Pro 615 620 625			3012
AAT AAC CCG GGA CGT AAC TAT GAT ATT TAC GAT CCT TTA GCT TTA GAT Asn Asn Pro Gly Arg Asn Tyr Asp Ile Tyr Asp Pro Leu Ala Leu Asp 630 635 640			3060
TTA GAT GGA GAT GGG CTT GAA ACC GTG TCG ATG AAC GGG CGA CAA GGC Leu Asp Gly Asp Gly Leu Glu Thr Val Ser Met Asn Gly Arg Gln Gly 645 650 655			3108
GCG TTA TTC GAT CAT GAA GGA AAA GGT ATT CGT ACC GCA ACG GGC TGG Ala Leu Phe Asp His Glu Gly Lys Gly Ile Arg Thr Ala Thr Gly Trp 660 665 670 675			3156
CTC GCT GCG GAT GAC GGT TTT TTA GTG TTA GAT CGT AAC CAA GAC GGC Leu Ala Ala Asp Asp Gly Phe Leu Val Leu Asp Arg Asn Gln Asp Gly 680 685 690			3204
ATT ATT AAT GAT ATA AGC GAG TTA TTT AGT AAT AAA AAT CAA CTT TCC Ile Ile Asn Asp Ile Ser Glu Leu Phe Ser Asn Lys Asn Gln Leu Ser 695 700 705			3252
GAC GGC AGT ATT TCT GCA CAC GGT TTT GCG ACA TTA GCC GAT TTG GAT Asp Gly Ser Ile Ser Ala His Gly Phe Ala Thr Leu Ala Asp Leu Asp 710 715 720			3300
ACA AAC CAA GAT CAG CGT ATC GAC CAA AAT GAT AAG CTG TTT TCT AAA Thr Asn Gln Asp Gln Arg Ile Asp Gln Asn Asp Lys Leu Phe Ser Lys 725 730 735			3348
CTC CAA ATT TGG CGG GAT TTA AAT CAA AAC GGT TTT AGT GAA GCG AAT Leu Gln Ile Trp Arg Asp Leu Asn Gln Asn Gly Phe Ser Glu Ala Asn 740 745 750 755			3396
GAG CTG TTT AGC TTA GAA AGT TTG AAT ATT AAA TCT TTA CAT ACC GCC Glu Leu Phe Ser Leu Glu Ser Leu Asn Ile Lys Ser Leu His Thr Ala 760 765 770			3444
TAT GAA GAG CGT AAT GAT TTT CTA GCG GGC AAT AAT ATC CTT GCT CAG Tyr Glu Glu Arg Asn Asp Phe Leu Ala Gly Asn Asn Ile Leu Ala Gln 775 780 785			3492
CTT GGG AAG TAT GAA AAA ACG GAC GGT ACT TTT GCA CAA ATG GGC GAT Leu Gly Lys Tyr Glu Lys Thr Asp Gly Thr Phe Ala Gln Met Gly Asp 790 795 800			3540
TTA AAT TTC AGT TTT AAC CCG TTT TAT AGC CGA TTT ACC GAA GCG TTA Leu Asn Phe Ser Phe Asn Pro Phe Tyr Ser Arg Phe Thr Glu Ala Leu 805 810 815			3588

CGG	GTT	CGG	GAT	TTG	CGT	GAA	GCC	GCC	GCA	CTT	TCT	GAG	GAG	TTG	GCT	3684
Arg	Val	Arg	Asp	Leu	Arg	Glu	Ala	Ala	Ala	Leu	Ser	Glu	Glu	Leu	Ala	
				840					845					850		
GCT	TTA	TTA	CAA	CAG	TAC	ACT	AAG	GCC	TCC	GAT	TTT	CAG	GCA	CAA	CGA	3732
Ala	Leu	Leu	Gln	Gln	Tyr	Thr	Lys	Ala	Ser	Asp	Phe	Gln	Ala	Gln	Arg	
			855					860					865			
GAA	TTA	TTG	CCT	GCC	ATT	TTA	GAT	AAA	TGG	GCG	GCA	ACG	GAT	TTA	CAG	3780
Glu	Leu	Leu	Pro	Ala	Ile	Leu	Asp	Lys	Trp	Ala	Ala	Thr	Asp	Leu	Gln	
		870					875					880				
TAT	CAA	CAT	TAT	GAT	AAA	ACA	TTA	CTT	AAA	ACG	GTA	GAA	AGT	ACC	GAT	3828
Tyr	Gln	His	Tyr	Asp	Lys	Thr	Leu	Leu	Lys	Thr	Val	Glu	Ser	Thr	Asp	
	885					890					895					
AGT	AGT	GCT	TCT	GTC	GTT	AGA	GTC	ACG	CCT	TCT	CAA	TTA	AGT	AGT	ATA	3876
Ser	Ser	Ala	Ser	Val	Val	Arg	Val	Thr	Pro	Ser	Gln	Leu	Ser	Ser	Ile	
900					905					910					915	
CGC	AAT	GCA	AAG	CAT	GAT	CCT	ACC	GTT	ATG	CAA	AAC	TTT	GAA	CAG	AGT	3924
Arg	Asn	Ala	Lys	His	Asp	Pro	Thr	Val	Met	Gln	Asn	Phe	Glu	Gln	Ser	
				920					925					930		
AAG	GCA	AAA	ATT	GCG	ACT	TTA	AAT	TCG	CTC	TAC	GGG	TTA	AAT	ATC	GAT	3972
Lys	Ala	Lys	Ile	Ala	Thr	Leu	Asn	Ser	Leu	Tyr	Gly	Leu	Asn	Ile	Asp	
			935					940					945			
CAA	CTT	TAT	TAC	ACG	ACG	GAT	AAA	GAC	ATT	CGC	TAT	ATT	ACT	GAT	AAA	4020
Gln	Leu	Tyr	Tyr	Thr	Thr	Asp	Lys	Asp	Ile	Arg	Tyr	Ile	Thr	Asp	Lys	
		950					955					960				
GTG	AAT	AAT	ATG	TAT	CAA	ACA	ACC	GTA	GAA	CTT	GCC	TAC	CGT	TCT	TTA	4068
Val	Asn	Asn	Met	Tyr	Gln	Thr	Thr	Val	Glu	Leu	Ala	Tyr	Arg	Ser	Leu	
	965					970					975					
CTT	TTA	CAA	ACG	CGT	TTG	AAG	AAA	TAT	GTT	TAT	AGC	GTT	AAT	GCG	AAA	4116
Leu	Leu	Gln	Thr	Arg	Leu	Lys	Lys	Tyr	Val	Tyr	Ser	Val	Asn	Ala	Lys	
980					985					990					995	
CAA	TTC	GAA	GGG	AAA	TGG	GTA	ACC	GAT	TAT	TCT	CGT	ACT	GAA	GCC	TTA	4164
Gln	Phe	Glu	Gly	Lys	Trp	Val	Thr	Asp	Tyr	Ser	Arg	Thr	Glu	Ala	Leu	
				1000					1005					1010		
TTT	AAC	TCT	ACT	TTT	AAA	CAA	TCG	CCT	GAA	AAT	GCA	TTA	TAT	GAT	TTA	4212
Phe	Asn	Ser	Thr	Phe	Lys	Gln	Ser	Pro	Glu	Asn	Ala	Leu	Tyr	Asp	Leu	
			1015					1020					1025			
AGC	GAA	TAC	CTT	TCT	TTC	TTT	AAC	GAT	CCT	ACG	GAA	TGG	AAA	GAA	GGG	4260
Ser	Glu	Tyr	Leu	Ser	Phe	Phe	Asn	Asp	Pro	Thr	Glu	Trp	Lys	Glu	Gly	
		1030					1035					1040				
CTA	TTA	CTG	TTA	AGC	CGT	TAT	ATA	GAT	TAT	GCT	AAA	GCA	CAA	GGA	TTT	4308
Leu	Leu	Leu	Leu	Ser	Arg	Tyr	Ile	Asp	Tyr	Ala	Lys	Ala	Gln	Gly	Phe	
		1045				1050					1055					
TAT	GAA	AAC	TGG	GCG	GCT	ACT	TCT	AAC	TTA	ACT	ATT	GCC	CGT	TTA	AGA	4356
Tyr	Glu	Asn	Trp	Ala	Ala	Thr	Ser	Asn	Leu	Thr	Ile	Ala	Arg	Leu	Arg	
1060					1065					1070					1075	
GAG	GCT	GGA	GTA	ATT	TTT	GCA	GAA	TCG	ACG	GAT	TTA	AAA	GGC	GAT	GAA	4404
Glu	Ala	Gly	Val	Ile	Phe	Ala	Glu	Ser	Thr	Asp	Leu	Lys	Gly	Asp	Glu	
				1080					1085					1090		
AAA	AAT	AAT	ATT	TTG	TTA	GGT	AGC	CAA	AAA	GAT	AAT	AAC	TTA	TCG	GGT	4452
Lys	Asn	Asn	Ile	Leu	Leu	Gly	Ser	Gln	Lys	Asp	Asn	Asn	Leu	Ser	Gly	
			1095					1100					1105			
AGT	GCA	GGT	GAT	GAT	CTA	CTT	ATC	GGC	GGA	GAG	GGT	AAT	GAT	ACG	TTA	4500
Ser	Ala	Gly	Asp	Asp	Leu	Leu	Ile	Gly	Gly	Glu	Gly	Asn	Asp	Thr	Leu	
		1110					1115					1120				
AAA	GGC	AGC	TAC	GGT	GCA	GAC	ACC	TAT	ATC	TTT	AGC	AAA	GGA	CAC	GGA	4548
Lys	Gly	Ser	Tyr	Gly	Ala	Asp	Thr	Tyr	Ile	Phe	Ser	Lys	Gly	His	Gly	
	1125					1130					1135					
CAG	GAT	ATC	GTT	TAT	GAA	GAT	ACC	AAT	AAT	GAT	AAC	CGC	GCA	AGA	GAT	4596
Gln	Asp	Ile	Val	Tyr	Glu	Asp	Thr	Asn	Asn	Asp	Asn	Arg	Ala	Arg	Asp	
1140					1145					1150					1155	
ATC	GAC	ACC	TTA	AAA	TTT	ACC	GAT	GTG	AAT	TAT	GCG	GAA	GTG	AAG	TTT	4644
Ile	Asp	Thr	Leu	Lys	Phe	Thr	Asp	Val	Asn	Tyr	Ala	Glu	Val	Lys	Phe	
				1160					1165					1170		

Arg	Arg	Val	Asp	Asn	Asp	Leu	Met	Leu	Phe	Gly	Tyr	His	Asp	Thr	Asp	
			1175					1180					1185			
TCG	GTC	ACG	GTA	AAA	TCC	TTC	TAC	AGC	CAT	GTA	GAT	TAT	CAA	TTT	GAC	4740
Ser	Val	Thr	Val	Lys	Ser	Phe	Tyr	Ser	His	Val	Asp	Tyr	Gln	Phe	Asp	
			1190				1195					1200				
AAA	TTG	GAG	TTT	GCT	GAC	CGC	AGT	ATA	ACT	CGC	GAT	GAA	CTG	ATT	AAA	4788
Lys	Leu	Glu	Phe	Ala	Asp	Arg	Ser	Ile	Thr	Arg	Asp	Glu	Leu	Ile	Lys	
			1205				1210				1215					
GCA	GGG	CTT	CAT	CTA	TAC	GGC	ACC	GAT	GGC	AAT	GAT	GAT	ATA	AAG	GAT	4836
Ala	Gly	Leu	His	Leu	Tyr	Gly	Thr	Asp	Gly	Asn	Asp	Asp	Ile	Lys	Asp	
			1220		1225					1230					1235	
CAT	GCG	GAT	TGG	GAC	AGC	ATT	TTG	GAA	GGC	GGC	AAA	GGC	AAC	GAT	ATT	4884
His	Ala	Asp	Trp	Asp	Ser	Ile	Leu	Glu	Gly	Gly	Lys	Gly	Asn	Asp	Ile	
				1240					1245					1250		
CTA	AGA	GGT	GGC	TAC	GGT	GCG	GAC	ACC	TAT	ATC	TTT	AGC	AAA	GGA	CAC	4932
Leu	Arg	Gly	Gly	Tyr	Gly	Ala	Asp	Thr	Tyr	Ile	Phe	Ser	Lys	Gly	His	
				1255				1260					1265			
GGA	CAG	GAT	ATC	GTT	TAT	GAA	GAT	ACC	AAT	AAT	GAT	AAC	CGC	GCA	AGA	4980
Gly	Gln	Asp	Ile	Val	Tyr	Glu	Asp	Thr	Asn	Asn	Asp	Asn	Arg	Ala	Arg	
			1270				1275					1280				
GAT	ATC	GAC	ACC	TTA	AAA	TTT	ACT	GAT	GTG	AAT	TAT	GCG	GAA	GTG	AAA	5028
Asp	Ile	Asp	Thr	Leu	Lys	Phe	Thr	Asp	Val	Asn	Tyr	Ala	Glu	Val	Lys	
			1285				1290				1295					
TTC	CGA	CGA	GTA	GAT	AAT	GAC	TTA	ATG	TTA	TTC	GGT	TAT	CAT	GAT	ACG	5076
Phe	Arg	Arg	Val	Asp	Asn	Asp	Leu	Met	Leu	Phe	Gly	Tyr	His	Asp	Thr	
			1300		1305					1310					1315	
GAT	TCG	GTC	ACG	ATA	AAA	TCC	TTC	TAC	AAC	CAT	GTA	GAT	TAT	CAA	TTT	5124
Asp	Ser	Val	Thr	Ile	Lys	Ser	Phe	Tyr	Asn	His	Val	Asp	Tyr	Gln	Phe	
				1320					1325					1330		
GAC	AAA	TTG	GAA	TTT	GCT	GAC	CGC	AGT	ATA	ACT	CGT	GAT	GAA	CTA	GGT	5172
Asp	Lys	Leu	Glu	Phe	Ala	Asp	Arg	Ser	Ile	Thr	Arg	Asp	Glu	Leu	Gly	
			1335					1340					1345			
AAA	CAA	GGT	ATG	GCA	TTA	TTT	GGC	ACT	GAC	GGT	GAT	GAT	AAT	ATC	AAC	5220
Lys	Gln	Gly	Met	Ala	Leu	Phe	Gly	Thr	Asp	Gly	Asp	Asp	Asn	Ile	Asn	
			1350				1355					1360				
GAC	TGG	GGA	CGT	AAC	TCG	GTG	ATT	GAT	GCC	GGT	GCG	GGT	AAT	GAT	ACG	5268
Asp	Trp	Gly	Arg	Asn	Ser	Val	Ile	Asp	Ala	Gly	Ala	Gly	Asn	Asp	Thr	
			1365			1370					1375					
GTT	AAT	GGC	GGT	AAT	GGC	GAT	GAC	ACC	CTC	ATC	GGC	GGC	AAA	GGT	AAT	5316
Val	Asn	Gly	Gly	Asn	Gly	Asp	Asp	Thr	Leu	Ile	Gly	Gly	Lys	Gly	Asn	
			1380		1385					1390					1395	
GAT	ATT	CTA	AGA	GGT	GGC	TAC	GGT	GCG	GAC	ACC	TAT	ATC	TTT	AGC	AAA	5364
Asp	Ile	Leu	Arg	Gly	Gly	Tyr	Gly	Ala	Asp	Thr	Tyr	Ile	Phe	Ser	Lys	
				1400				1405						1410		
GGA	CAC	GGA	CAG	GAT	ATC	GTT	TAT	GAA	GAT	ACC	AAT	AAT	GAT	AAC	CGC	5412
Gly	His	Gly	Gln	Asp	Ile	Val	Tyr	Glu	Asp	Thr	Asn	Asn	Asp	Asn	Arg	
			1415				1420					1425				
GCA	AGA	GAT	ATC	GAC	ACC	TTA	AAA	TTT	ACC	GAT	GTG	AAT	TAT	GCG	GAA	5460
Ala	Arg	Asp	Ile	Asp	Thr	Leu	Lys	Phe	Thr	Asp	Val	Asn	Tyr	Ala	Glu	
			1430				1435					1440				
GTG	AAA	TTC	CGA	CGA	GTA	GAT	AAT	GAC	TTA	ATG	TTA	TTC	GGT	TAT	CAT	5508
Val	Lys	Phe	Arg	Arg	Val	Asp	Asn	Asp	Leu	Met	Leu	Phe	Gly	Tyr	His	
			1445			1450					1455					
GAT	ACG	GAT	TCG	GTC	ACG	GTA	AAA	TCC	TTC	TAC	AGC	CAT	GTA	GAT	TAT	5556
Asp	Thr	Asp	Ser	Val	Thr	Val	Lys	Ser	Phe	Tyr	Ser	His	Val	Asp	Tyr	
				1460	1465					1470					1475	
CAA	TTT	GAC	AAA	TTG	GAG	TTT	GCT	GAC	CGC	AGT	ATA	ACT	CGC	GAT	GAA	5604
Gln	Phe	Asp	Lys	Leu	Glu	Phe	Ala	Asp	Arg	Ser	Ile	Thr	Arg	Asp	Glu	
				1480				1485						1490		
CTG	ATT	AAA	GCA	GGG	CTT	CAT	CTA	TAC	GGC	ACC	GAT	GGC	AAT	GAT	GAT	5652
Leu	Ile	Lys	Ala	Gly	Leu	His	Leu	Tyr	Gly	Thr	Asp	Gly	Asn	Asp	Asp	
			1495					1500					1505			

1510	1515	1520	
AAC GAT ATT CTA AGA GGT GGC TAC GGT GCG GAC ACC TAT ATC TTT AGC Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser 1525 1530 1535			5748
AAA GGA CAC GGA CAG GAT ATC GTT TAT GAA GAT ACC AAT AAT GAT AAC Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn 1540 1545 1550 1555			5796
CGA GCA AGA GAT ATC GAC ACC TTA AAA TTT ACT GAT GTG AAT TAT GCG Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala 1560 1565 1570			5844
GAA GTG AAA TTC CGA CGA GTA GAT AAT GAC TTA ATG TTA TTC GGT TAT Glu Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr 1575 1580 1585			5892
CAT GAT ACG GAT TCG GTC ACG ATA AAA TCC TTC TAC AAC CAT GTA GAT His Asp Thr Asp Ser Val Thr Ile Lys Ser Phe Tyr Asn His Val Asp 1590 1595 1600			5940
TAT CAA TTT GAC AAA TTG GAA TTT GCT GAC CGC AGT ATA ACT CGT GAT Tyr Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp 1605 1610 1615			5988
GAA CTA GGT AAA CAA GGT ATG GCA TTA TTT GGC ACT GAC GGT GAT GAT Glu Leu Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp 1620 1625 1630 1635			6036
AAT ATC AAC GAC TGG GGA CGT AAC TCG GTG ATT GAT GCC GGT GCG GGT Asn Ile Asn Asp Trp Gly Arg Asn Ser Val Ile Asp Ala Gly Ala Gly 1640 1645 1650			6084
AAT GAT ACG GTT AAT GGC GGT AAT GGC GAT GAC ACC CTC ATC GGC GGC Asn Asp Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly 1655 1660 1665			6132
AAA GGT AAT GAT ATT CTA AGA GGT GGC TAC GGT GCG GAC ACC TAT ATC Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile 1670 1675 1680			6180
TTT AGC AAA GGA CAC GGA CAG GAT ATC GTT TAT GAA GAT ACC AAT AAT Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn 1685 1690 1695			6228
GAT AAC CGC GCA AGA GAT ATC GAC ACC TTA AAA TTT ACT GAT ATT AAT Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Ile Asn 1700 1705 1710 1715			6276
TTA TCC GAA CTT TGG TTT AGC CGA GAA AAT AAC GAT TTG ATT ATT AAA Leu Ser Glu Leu Trp Phe Ser Arg Glu Asn Asn Asp Leu Ile Ile Lys 1720 1725 1730			6324
TCA TTA TTA AGT GAG GAT AAA GTC ACG GTT CAA AAT TGG TAT TCA CAC Ser Leu Leu Ser Glu Asp Lys Val Thr Val Gln Asn Trp Tyr Ser His 1735 1740 1745			6372
CAA GAT CAT AAA ATA GAA AAT ATT CGT TTA TCG AAT GAG CAA ACG TTG Gln Asp His Lys Ile Glu Asn Ile Arg Leu Ser Asn Glu Gln Thr Leu 1750 1755 1760			6420
GTG AGC ACT CAG GTG GAG AAG ATG GTT GAG TCG ATG GCC GGC TTT GCT Val Ser Thr Gln Val Glu Lys Met Val Glu Ser Met Ala Gly Phe Ala 1765 1770 1775			6468
CAG AAG CAC GGA GGA GAG ATA TCT CTT GTG TCG CTT GAA GAG GTA AAA Gln Lys His Gly Gly Glu Ile Ser Leu Val Ser Leu Glu Glu Val Lys 1780 1785 1790 1795			6516
CAA TAT ATC AAT AGC TTA ACA GCT GCT TTA TAA CATAACGAAAG AAATCGGCAC Gln Tyr Ile Asn Ser Leu Thr Ala Ala Leu * 1800 1805			6569
AGTTTTTTTTG AACTGTGCCG ATTTGATTTT AGTGTAAGAA TATAGCCTGA TTTTAAGAAA			6629
TTTACTCTTG GCTAATAACT ATTTCCCATTTTATAAGTTA TTGACGGATG GTTTTATCAA			6689
ATATGAGATC AAATCTTATT TTAAATTCGC TTTCCATTAA GCGATAT			6736

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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Ile Asp Met Pro Pro Gly Thr Gly Asp Ile Gln Leu Thr Leu Ser Gln
 1          5          10          15
Gln Ile Pro Val Thr Gly Ala Val Val Val Thr Thr Pro Gln Asp Ile
          20          25          30
Ala Leu Leu Asp Ala Val Lys Gly Ile Ser Met Phe Gln Lys Val Ser
          35          40          45
Val Pro Val Leu Gly Ile Ile Glu Asn Met Ser Val His Ile Cys Gln
          50          55          60
Asn Cys Gly His His Glu Asp Ile Phe Gly Thr Gly Gly Ala Glu Lys
 65          70          75          80
Val Ala Lys Lys Tyr Gly Thr Lys Val Leu Gly Gln Met Pro Leu His
          85          90          95
Ile Arg Leu Arg Gln Asp Leu Asp Ala Gly Thr Pro Thr Val Val Ala
          100          105          110
Ala Pro Glu His Glu Thr Ser Arg Ala Tyr Ile Glu Leu Ala Ala Lys
          115          120          125
Val Ala Ser Glu Leu Tyr Trp Gln Gly Ser Val Ile Pro Ser Glu Ile
          130          135          140
Met Ile Arg Glu Val Lys *
145          150

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(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1806 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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Met Thr Lys Leu Thr Met Gln Asp Val Thr Asn Leu Tyr Leu Tyr Lys
 1          5          10          15
Thr Lys Thr Leu Pro Lys Asp Arg Leu Asp Asp Ser Leu Ile Ser Glu
          20          25          30
Ile Gly Lys Gly Asp Asp Asp Ile Asp Arg Lys Glu Phe Met Val Gly
          35          40          45
Pro Gly Arg Phe Val Thr Ala Asp Asn Phe Ser Val Val Arg Asp Phe
          50          55          60
Phe Asn Ala Gly Lys Ser Arg Ile Ile Ala Pro Gln Val Pro Pro Ile
 65          70          75          80
Arg Ser Gln Gln Glu Lys Ile Leu Val Gly Leu Lys Pro Gly Lys Tyr
          85          90          95
Ser Lys Ala Gln Ile Leu Glu Met Leu Gly Tyr Thr Lys Gly Gly Glu
          100          105          110
Val Val Asn Gly Met Phe Ala Gly Glu Val Gln Thr Leu Gly Phe Tyr
          115          120          125
Asp Asp Gly Lys Gly Asp Leu Leu Glu Arg Ala Tyr Ile Trp Asn Thr
          130          135          140
Thr Gly Phe Lys Met Ser Asp Asn Ala Phe Phe Val Ile Glu Glu Ser
          145          150          155          160
Gly Lys Arg Tyr Ile Glu Asn Phe Gly Ile Glu Pro Leu Gly Lys Gln
          165          170          175
Glu Asp Phe Asp Phe Val Gly Gly Phe Trp Ser Asn Leu Val Asn Arg
          180          185          190

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Leu Asn Phe Thr Gly Glu Val Glu Thr Tyr Thr Leu Asp Glu Thr Arg
 210 215 220
 Phe Lys Ala Glu Ala Ala Lys Lys Ser His Trp Ser Leu Val Asn Ala
 225 230 235 240
 Ala Lys Val Tyr Gly Gly Leu Asp Gln Ile Ile Lys Lys Leu Trp Asp
 245 250 255
 Ser Gly Ser Ile Lys His Leu Tyr Gln Asp Lys Asp Thr Gly Lys Leu
 260 265 270
 Lys Pro Ile Ile Tyr Gly Thr Ala Gly Asn Asp Ser Lys Ile Glu Gly
 275 280 285
 Thr Lys Ile Thr Arg Arg Ile Ala Gly Lys Glu Val Thr Leu Asp Ile
 290 295 300
 Ala Asn Gln Lys Ile Glu Lys Gly Val Leu Glu Lys Leu Gly Leu Ser
 305 310 315 320
 Val Ser Gly Ser Asp Ile Ile Lys Leu Leu Phe Gly Ala Leu Thr Pro
 325 330 335
 Thr Leu Asn Arg Met Leu Leu Ser Gln Leu Ile Gln Ser Phe Ser Asp
 340 345 350
 Ser Leu Ala Lys Leu Asp Asn Pro Leu Ala Pro Tyr Thr Lys Asn Gly
 355 360 365
 Val Val Tyr Val Thr Gly Lys Gly Asn Asp Val Leu Lys Gly Thr Glu
 370- 375 380
 His Glu Asp Leu Phe Leu Gly Gly Glu Gly Asn Asp Thr Tyr Tyr Ala
 385 390 395 400
 Arg Val Gly Asp Thr Ile Glu Asp Ala Asp Gly Lys Gly Lys Val Tyr
 405 410 415
 Phe Val Arg Glu Lys Gly Val Pro Lys Ala Asp Pro Lys Arg Val Glu
 420 425 430
 Phe Ser Glu Tyr Ile Thr Lys Glu Glu Ile Lys Glu Val Glu Lys Gly
 435 440 445
 Leu Leu Thr Tyr Ala Val Leu Glu Asn Tyr Asn Trp Glu Glu Lys Thr
 450 455 460
 Ala Thr Phe Ala His Ala Thr Met Leu Asn Glu Leu Phe Thr Asp Tyr
 465 470 475 480
 Thr Asn Tyr Arg Tyr Glu Val Lys Gly Leu Lys Leu Pro Ala Val Lys
 485 490 495
 Lys Leu Lys Ser Pro Leu Val Glu Phe Thr Ala Asp Leu Leu Thr Val
 500 505 510
 Thr Pro Ile Asp Glu Asn Gly Lys Ala Leu Ser Glu Lys Ser Ile Thr
 515 520 525
 Val Lys Asn Phe Lys Asn Gly Asp Leu Gly Ile Arg Leu Leu Asp Pro
 530 535 540
 Asn Ser Tyr Tyr Tyr Phe Leu Glu Gly Gln Asp Thr Gly Phe Tyr Gly
 545 550 555 560
 Pro Ala Phe Tyr Ile Glu Arg Lys Asn Gly Gly Gly Ala Lys Asn Asn
 565 570 575
 Ser Ser Gly Ala Gly Asn Ser Lys Asp Trp Gly Gly Asn Gly His Gly
 580 585 590
 Asn His Arg Asn Asn Ala Ser Asp Leu Asn Lys Pro Asp Gly Asn Asn
 595 600 605
 Gly Asn Asn Gln Asn Asn Gly Ser Asn Gln Asp Asn His Ser Asp Val
 610 615 620
 Asn Ala Pro Asn Asn Pro Gly Arg Asn Tyr Asp Ile Tyr Asp Pro Leu
 625 630 635 640
 Ala Leu Asp Leu Asp Gly Asp Gly Leu Glu Thr Val Ser Met Asn Gly
 645 650 655

660							665							670						
Thr	Gly	Trp	Leu	Ala	Ala	Asp	Asp	Gly	Phe	Leu	Val	Leu	Asp	Arg	Asn					
675		680		685		690		695		700		705		710						
Gln	Asp	Gly	Ile	Ile	Asn	Asp	Ile	Ser	Glu	Leu	Phe	Ser	Asn	Lys	Asn					
695		700		705		710		715		720		725		730						
Gln	Leu	Ser	Asp	Gly	Ser	Ile	Ser	Ala	His	Gly	Phe	Ala	Thr	Leu	Ala					
705		710		715		720		725		730		735		740						
Asp	Leu	Asp	Thr	Asn	Gln	Asp	Gln	Arg	Ile	Asp	Gln	Asn	Asp	Lys	Leu					
715		720		725		730		735		740		745		750						
Phe	Ser	Lys	Leu	Gln	Ile	Trp	Arg	Asp	Leu	Asn	Gln	Asn	Gly	Phe	Ser					
725		730		735		740		745		750		755		760						
Glu	Ala	Asn	Glu	Leu	Phe	Ser	Leu	Glu	Ser	Leu	Asn	Ile	Lys	Ser	Leu					
735		740		745		750		755		760		765		770						
His	Thr	Ala	Tyr	Glu	Glu	Arg	Asn	Asp	Phe	Leu	Ala	Gly	Asn	Asn	Ile					
745		750		755		760		765		770		775		780						
Leu	Ala	Gln	Leu	Gly	Lys	Tyr	Glu	Lys	Thr	Asp	Gly	Thr	Phe	Ala	Gln					
765		770		775		780		785		790		795		800						
Met	Gly	Asp	Leu	Asn	Phe	Ser	Phe	Asn	Pro	Phe	Tyr	Ser	Arg	Phe	Thr					
785		790		795		800		805		810		815		820						
Glu	Ala	Leu	Asn	Leu	Thr	Glu	Gln	Gln	Arg	Arg	Thr	Ile	Asn	Leu	Thr					
795		800		805		810		815		820		825		830						
Gly	Thr	Gly	Arg	Val	Arg	Asp	Leu	Arg	Glu	Ala	Ala	Ala	Leu	Ser	Glu					
805		810		815		820		825		830		835		840						
Glu	Leu	Ala	Ala	Leu	Leu	Gln	Gln	Tyr	Thr	Lys	Ala	Ser	Asp	Phe	Gln					
825		830		835		840		845		850		855		860						
Ala	Gln	Arg	Glu	Leu	Leu	Pro	Ala	Ile	Leu	Asp	Lys	Trp	Ala	Ala	Thr					
845		850		855		860		865		870		875		880						
Asp	Leu	Gln	Tyr	Gln	His	Tyr	Asp	Lys	Thr	Leu	Leu	Lys	Thr	Val	Glu					
865		870		875		880		885		890		895		900						
Ser	Thr	Asp	Ser	Ser	Ala	Ser	Val	Val	Arg	Val	Thr	Pro	Ser	Gln	Leu					
885		890		895		900		905		910		915		920						
Ser	Ser	Ile	Arg	Asn	Ala	Lys	His	Asp	Pro	Thr	Val	Met	Gln	Asn	Phe					
895		900		905		910		915		920		925		930						
Glu	Gln	Ser	Lys	Ala	Lys	Ile	Ala	Thr	Leu	Asn	Ser	Leu	Tyr	Gly	Leu					
905		910		915		920		925		930		935		940						
Asn	Ile	Asp	Gln	Leu	Tyr	Tyr	Thr	Thr	Asp	Lys	Asp	Ile	Arg	Tyr	Ile					
925		930		935		940		945		950		955		960						
Thr	Asp	Lys	Val	Asn	Asn	Met	Tyr	Gln	Thr	Thr	Val	Glu	Leu	Ala	Tyr					
945		950		955		960		965		970</										

Asp Thr Leu Lys Gly Ser Tyr Gly Ala Asp Thr Tyr Ile Phe Ser Lys
 1125 1130 1135
 Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn Arg
 1140 1145 1150
 Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala Glu
 1155 1160 1165
 Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr His
 1170 1175 1180
 Asp Thr Asp Ser Val Thr Val Lys Ser Phe Tyr Ser His Val Asp Tyr
 1185 1190 1195 1200
 Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp Glu
 1205 1210 1215
 Leu Ile Lys Ala Gly Leu His Leu Tyr Gly Thr Asp Gly Asn Asp Asp
 1220 1225 1230
 Ile Lys Asp His Ala Asp Trp Asp Ser Ile Leu Glu Gly Gly Lys Gly
 1235 1240 1245
 Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile Phe Ser
 1250 1255 1260
 Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn Asp Asn
 1265 1270 1275 1280
 Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn Tyr Ala
 1285 1290 1295
 Glu Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe Gly Tyr
 1300 1305 1310
 His Asp Thr Asp Ser Val Thr Ile Lys Ser Phe Tyr Asn His Val Asp
 1315 1320 1325
 Tyr Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr Arg Asp
 1330 1335 1340
 Glu Leu Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp Gly Asp Asp
 1345 1350 1355 1360
 Asn Ile Asn Asp Trp Gly Arg Asn Ser Val Ile Asp Ala Gly Ala Gly
 1365 1370 1375
 Asn Asp Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu Ile Gly Gly
 1380 1385 1390
 Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr Ile
 1395 1400 1405
 Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn Asn
 1410 1415 1420
 Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val Asn
 1425 1430 1435 1440
 Tyr Ala Glu Val Lys Phe Arg Arg Val Asp Asn Asp Leu Met Leu Phe
 1445 1450 1455
 Gly Tyr His Asp Thr Asp Ser Val Thr Val Lys Ser Phe Tyr Ser His
 1460 1465 1470
 Val Asp Tyr Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile Thr
 1475 1480 1485
 Arg Asp Glu Leu Ile Lys Ala Gly Leu His Leu Tyr Gly Thr Asp Gly
 1490 1495 1500
 Asn Asp Asp Ile Lys Asp His Ala Asp Trp Asp Ser Ile Leu Glu Gly
 1505 1510 1515 1520
 Gly Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp Thr Tyr
 1525 1530 1535
 Ile Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp Thr Asn
 1540 1545 1550
 Asn Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr Asp Val
 1555 1560 1565

1570	1575	1580
Phe Gly Tyr His Asp Thr Asp Ser Val Thr Ile Lys Ser Phe Tyr Asn 1585 1590 1595 1600		
His Val Asp Tyr Gln Phe Asp Lys Leu Glu Phe Ala Asp Arg Ser Ile 1605 1610 1615		
Thr Arg Asp Glu Leu Gly Lys Gln Gly Met Ala Leu Phe Gly Thr Asp 1620 1625 1630		
Gly Asp Asp Asn Ile Asn Asp Trp Gly Arg Asn Ser Val Ile Asp Ala 1635 1640 1645		
Gly Ala Gly Asn Asp Thr Val Asn Gly Gly Asn Gly Asp Asp Thr Leu 1650 1655 1660		
Ile Gly Gly Lys Gly Asn Asp Ile Leu Arg Gly Gly Tyr Gly Ala Asp 1665 1670 1675 1680		
Thr Tyr Ile Phe Ser Lys Gly His Gly Gln Asp Ile Val Tyr Glu Asp 1685 1690 1695		
Thr Asn Asn Asp Asn Arg Ala Arg Asp Ile Asp Thr Leu Lys Phe Thr 1700 1705 1710		
Asp Ile Asn Leu Ser Glu Leu Trp Phe Ser Arg Glu Asn Asn Asp Leu 1715 1720 1725		
Ile Ile Lys Ser Leu Leu Ser Glu Asp Lys Val Thr Val Gln Asn Trp 1730 1735 1740		
Tyr Ser His Gln Asp His Lys Ile Glu Asn Ile Arg Leu Ser Asn Glu 1745 1750 1755 1760		
Gln Thr Leu Val Ser Thr Gln Val Glu Lys Met Val Glu Ser Met Ala 1765 1770 1775		
Gly Phe Ala Gln Lys His Gly Gly Glu Ile Ser Leu Val Ser Leu Glu 1780 1785 1790		
Glu Val Lys Gln Tyr Ile Asn Ser Leu Thr Ala Ala Leu *	1800	1805